Smart dendrimers: Synergizing the targeting of anticancer bioactives

Manuscript Details

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Title	Smart dendrimers: Synergizing the targeting of anticancer bioactives
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Abstract

Optimization of biological performance of a carrier in cancer drug delivery depend on the targeting potential of the delivery system and its ability to control the drug release precisely. Dendrimers has emerged as a potential carrier of anticancer drugs due to some unique properties such as mono-dispersity, defined structure, amenability for functionalization using diverse ligands and its low-nanometer size. The dendrimers could be decorated to make them smart enough to carry the drug to the desired locus and release it in a controlled manner. The introduction of stimuli responsive functionality on dendrimers allows the release of payloads in response to a specific trigger only. These triggers could be endogenous in nature (acid, enzyme, and redox potentials) or it could be applied externally (light and temperature). These smart functionalities synergize the targeting of dendrimers and enable dendrimer-based anticancer drug delivery more efficient and safer. This review highlights the potential of stimuli responsive strategy for the controlled release of anticancer drug from dendritic assemblies.

Keywords	Dendrimers; Drug release; Cancer; Drug targeting; Toxicity; Anticancer drug

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Submission Files Included in this PDF

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No data was used for the research described in the article

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Title: Nanoneuromedicine: An effective vista for management of neurodegenerative disorder

Journal: Journal of Drug Delivery Science and Technology

To,
Professor Müllertz
Associate Editor
Journal of Drug Delivery Science and Technology

Subject: Regarding submission of revised manuscript entitled "Smart dendrimers: Synergizing the targeting of anticancer bioactives"

Dear Prof. Müllertz,

With reference to your e-mail dated 10th March 2019, we are happy to hear that our manuscript has been reviewed by potential reviewers and they concluded to accept our paper subjected to major revisions. As per reviewer suggestions, we have cautiously gone through the comments regarding required changes in the manuscript (please see "Response to reviewers" file for details). We have thoroughly revised the manuscript by highlighting the text in red color to address the raised concerns. Hope you will find revised manuscript suitable for publication.

Thanking you in anticipation and a favorable response.

Kindly acknowledge

Best Regards

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RESPONSES TO THE REVIEWERS COMMENTS

Reviewer 1

This manuscript introduced the dendritic polymers-based stimuli-responsive drug delivery systems for cancer treatments. The dendritic polymer have been studied well and showed great potential as safe and efficient carriers. Additionally, a number of research groups still focus on this kind of polymer. Therefore, this manuscript is interesting and useful for this field. Some suggesting may be considered to improve this manuscript.

Comment 1: The key words "dendrimer" and "functionalized dendrimer" is repeated, one of them may be removed, and a new key word may be added.

Reply: Complied; a new keyword has been added.

Comment 2: As dendrimers have been studied well, and a number of reviews have been reported. The authors may listed those reviews to the revised manuscript, including Advanced Drug Delivery Reviews 2005, 57, 2215-2237; Biotechnology Advances 2014, 32, 818-830; Progress in Polymer Science 2015, 44, 1-27; Drug Discovery Today 2017, 22, 314-326; Drug Discovery Today 2018, 23, 300-314.

Reply: Thanks to honored reviewer for the suggestion. The suggested reviews have been incorporated in the revised manuscript.

Comment 3: Dendrimer is one of kinds of dendritic polymers. One paragraph about the comparative discussions of the linear polymer, dendritic polymers and dendrimer may be added to the revised manuscript.

Reply: Complied; As per the suggestion of the honored reviewer the comparative discussion of the linear polymer, dendritic polymers and dendrimer has been added to the revised manuscript.

Comment 4: More relative studies on responsive polymer-based drug delivery may be added to the revise manuscript, including Biomaterials Science, 2019, DOI: 10.1039/c8bm01103f; Acta Biomaterialia, 2019, 84, 339-355; Journal of Biomedical Nanotechnology, 2017, 13, 1089-1096; Current Topics in Medicinal Chemistry, 2017, 17, 1542-1554; Biomaterials Science, 2018, 6, 2976-2986; Journal of Biomedical Nanotechnology 2017, 13(11), 1369-1385; ACS Applied Materials & Interfaces 2016, 8, 11765-11778; Int. J. Environ. Res. Public

Health 2018, 15(2), 338; Materials Science and Engineering: C 2018, 90, 713-727; Biomaterials 2014, 35(38), 10080-10092.

Reply: Complied; As per the suggestion of the honored reviewer the studies on responsive polymer-based drug delivery have been added to the revised manuscript.

Reviewer 2

There is no doubt that Saluja et al. chose an interesting topic for their review. This topic is relevant to the scope of the journal. The paper is good from the linguistic point of view and in this aspect it facilitates reading. However, the way the article is organized is a bit boring. Most paragraphs starts from "X. et al. synthesized/fabricated/developed", etc. It makes the review more a set of abstracts than the real review where the part of discussing the results, comparing them and drawing conclusions is important. The conclusion section is very short. The review as it is now is useful in terms of collecting a lot of examples in one place. However, I believe that there is potential for much more. I encourage Authors to rethink their article and, based on the impressive data that they collected so far, prepare the text that is more original.

Reply: Thanks for the appreciation. As per the suggestion of the honored reviewer, we have rethought and frame work of the studies has been revised. The conclusion section has also been elaborated.

Reviewer 3

The authors reviewed the advances of smart dendrimers for targeted delivery of anticancer drugs. In recent years, there are lots of review articles on this topic and thus the ones that can be published should be focused on most recent examples in this field. After reading the manuscript, I suggest its major revision before it can be published by Journal of Drug Delivery Science and Technology. The following are some detailed comments.

Comment 1: The figures in the manuscript are reproduced from the references and therefore the authors should first got the permissions from the publishers and give a citation on the figure captions.

Reply: Complied. The permission has been taken for reuse of the figures and due citation has been provided in caption. The Fig 1 has been modified in to a tabular form.

Comment 2: I suggest the authors provide high resolution figures, or they may redraw the figures according to the original ones in the revised manuscript.

Reply: Complied; high resolution figures have been provided in the revised manuscript.

Comment 3: How to prove the synergistic effect of these responsiveness in targeted cancer therapy? The authors emphasized this in the title and abstract. They may provide detailed examples to prove this issue in the review.

Reply: Dendrimers has emerged as unique polymeric globular nanoparticulate drug delivery system that could be judiciously utilized to tackle the deadliest disease cancer. The inimitable topographical molecular architect encompassing this class of delivery system could allow the delivery of varying nature of anticancer bioactives viz lipophilic or hydrophilic drugs and big macromolecules as proteins or RNA. The prospect of multifunctionality owing to multivalency, leads to decoration of their surface by different moieties for varying function to achieve a common goal and this could significantly enhance the efficacy of the transported bioactives. The conventional chemotherapy for cancer management exhibits a lack of selectivity and thus affecting healthy tissues. To realize selectivity, the dendrimers could be functionalized using moieties that would synergistically act to target the tumoral cells and release the payloads at the desired site. The approach of "cellular or secondary targeting" based on moieties that leads to ligand–receptor-mediated endocytosis or of "tertiary targeting" based on moieties that recognize internal organelles or the use of stimuli-responsive moieties that are responsible for release of bioactives under specific internal or external stimuli, in combination onto a single dendritic structure synergistically act to achieve selective targeting.

Comment 4: In the section of 4. Reduciton-sensitive dendrimers: the example on GSH-responsive dendrimers (J. Am. Chem. Soc., 2013, 135(26): 9805-9810; J. Am. Chem. Soc., 2012, 134(42): 17680-17687) should be discussed.

Reply: Complied.

Comment 5: In the section of 6. Temperature-senstive dendrimers: the example of Temperature responsive gene silencing by a smart polymer (Bioconjugate Chem., 2016, 27(3), 495-499) is suggested.

Reply: Complied.

Comment 6: The authors missed smart dendrimers in gene delivery in this review article, for example, fluorinated dendrimers in gene delivery (Nat. Commun., 2014, 5, 3053; Angew Chem Int Ed., 2015, 54(40): 11647-11651, also a critical review: Acta Polymerica Sinica, 2017, 8, 1234- 1245).

Reply: Complied.

Comment 7: The language should be improved before resubmission. Also, the authors should carefully edit the manuscript according to the requirements of JDDST.

Reply: Complied. As per the suggestion of the honored reviewer, the manuscript has been revised in relation to the English language with due assistance from a native English speaker and carefully edited the manuscript according to the requirements of JDDST.

Smart dendrimers: Synergizing the targeting of anticancer bioactives

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Abstract

Optimization of biological performance of a carrier in cancer drug delivery depends on the targeting potential of the delivery system and its ability to control the drug release precisely. Dendrimers has emerged as a potential carrier of anticancer drugs due to some unique properties such as mono-dispersity, defined structure, amenability for functionalization using diverse ligands and its low-nanometer size. The dendrimers could be decorated to make them smart enough to carry the drug to the desired locus and release it in a controlled manner. The introduction of stimuli responsive functionality on dendrimers allows the release of payloads in response to a specific trigger only. These triggers could be endogenous in nature (acid, enzyme, and redox potentials) or it could be applied externally (light and temperature). These smart functionalities synergize the targeting of dendrimers and enable dendrimer-based anticancer drug delivery more efficient and safer. This review highlights the potential of stimuli responsive strategy for the controlled release of anticancer drug from dendritic assemblies.

Keywords: Dendrimers; Drug release; Cancer; Drug targeting; Toxicity; Anticancer drug

1. Introduction

Optimizing the outcome of already existing drugs is currently the focusing area of research in view of improving physicochemical, biodistribution and pharmacokinetic properties of the drugs to enhance therapeutic benefit with minimal side-effects. The size manipulation at the molecular level could leads to new intrinsic properties and would be translated as technological innovations for improving the outcome of conventional systems. In field of medicine such technological innovations are termed as nanomedicine. The aim of nanomedicine is to diagnose a disease in its initial stage and to treat it rapidly and specifically, therefore, restrict long-term damage [1]. The field of nanomedicine could be prudently utilized in the management of the world's most deadly disease cancer which is responsible for about 22% of all human deaths annually from non-communicable diseases [2,3].

With hundreds of signaling pathways and multiple causes that respond differently to various treatments, cancer remains an ongoing challenge with enormous health and financial burdens on patients and society. The stage of the cancer is the decisive factor in opting the treatment regimen via surgery, radiation, chemotherapy, biological and hormone therapy. Nonetheless, chemotherapy remains the mainstay option for cancer management and depending on type of cancer and its advancement, it is used as adjuvant with surgery, radiation therapy, or biological therapy [4].

Non-selective biodistribution, low aqueous solubility with poor membrane permeability and rapid clearance, hypersensitivity reactions, and advent of multidrug resistance (MDR) are the major challenges in conventional chemotherapy [5]. Thus, a drug therapy that shows reproducibility in pharmacokinetic behavior and is target specific is sought. A stable, monodisperse, well-defined system could ensure the reproducibility in pharmacokinetic behavior

of a therapy *in-vivo*. This realization leads to the synthesis of hyperbranched polymers termed as dendrimers [6].

2. Dendrimers: An outline

Dendrimers, a class of artificial macromolecules, are nanosize, monodisperse, multivalent polymeric systems with well-defined structure. These unique exciting features ensure a pivotal role of dendrimers in the management of cancer. Structurally, a dendrimer has a treelike molecular construction and comprises of three main architectural components including a core, branching layers (termed as generations) consist of repeating units surrounding the internal core and a multivalent external surface. Generation number (G) signifies the number of focal points from the core towards the surface and is used to determine molecular weight and the number of terminal functional groups [7]. Drugs could either be encapsulated within dendrimers internal cavity or bound to their surfaces through hydrophobic, electrostatic or covalent interactions. The immense potential in this class of molecular construct leads to synthesis of various classes of dendrimers, such as poly(propylene imine) (PPI), poly(amidoamine) (PAMAM), chiral, amphiphilic, micellar, Tecto, Frechet and Janus dendrimers and are also commercially available [8].

Due to highly dense structure, perfect chemical definition and a well-defined number of surface functionalities, dendrimers could be adaptable to multifunctional modifications with valuable flexibility for grafting different chemical moieties on the surface, at the core, or within the structure. The most promising feature of dendrimers is their multi-valency, which presents multiple arrays of ligands to the target bearing multiple receptors. This leads to a greatly increased interface between the dendrimer and the target enhancing affinity and activity [9,10].

2.1 Comparative account of linear polymer, dendritic polymers and dendrimer

Dendritic polymers considered as branched polymeric architectures, are classified into random hyperbranched polymers, dendrigraft polymers, dendrons, and dendrimers based on degree of structural control [11]. In general, branched polymers have sophisticated topological structures and exciting physico-chemical and biological properties. In comparison to their linear analogues, branched polymers have three-dimensional globular structure, lower solution/ melting viscosity, smaller hydrodynamic radius, higher degree of functionality, increased encapsulation capabilities, enhanced solubility and minimal molecular entanglement. Owing to high functionality, dendritic structures, allows dense incorporation of drugs, imaging and targeting agents [12]. Monodisperse nature of dendrimers, in addition, provides reproducible pharmacokinetic behavior as compared to linear polymers, which are generally polydisperse and thus containing varying molecular weighed fractions among a given sample. Also, controlled globular shape of dendrimers other than entangled and coiled structures of linear polymers enhances their biological properties [13]. Furthermore, prospect of surface engineering due their high density of functionalities allow to tune their thermal, mechanical, rheological, solution properties (size, conformation, solubility), and biocompatibility. These features can further improve the biodistribution and pharmacokinetic profile, tendency of crossing biological barriers, blood circulation time and tumor penetration [14-16].

2.2 Toxicologial profile of dendrimers and remedies

The emergence of dendrimers is foresighted as solution to various biomedical difficulties due to distinct features like nanometric size, well-defined structure, narrow polydispersity and large number of surface groups. However, owing their toxic potential including hemolytic toxicity, cytotoxicity, immunogenicity and *in vivo* toxicity, the credentials of their clinical applications is limited. The toxicity of dendrimers is related to surface charge, generation and

size [17, 18]. Various approaches are being utilized to improve the therapeutic index of dendrimers including development of biodegradable and/or biocompatible, surface engineered dendrimers and use of dual drug delivery systems. The biodegradable dendrimers, generally composed by biodegradable repeat units that will eliminate metabolic pathways and thus will not accumulate, therefore considered as non-toxic [19]. Surface engineering masks the cationic charge of dendrimer surface either by neutralization of charge, for example PEGylation, acetylation, carbohydrate and peptide conjugation; or by introducing negative charge on the surface of dendrimers [10]. Dendrimer based hybrid nanocarrier is recognized as a recent approach to minimize the toxicity, improving dispersibility, biocompatibility, payloads and pharmacokinetics of dendrimers as compared to other carriers such as liposomes, nanoparticles, quantum-dots, carbon nanotubes and microsphere [20].

2.3 Dendrimer based drug targeting approach

Dendrimers has emerged as unique polymeric globular nanoparticulate drug delivery system that could be judiciously utilized to tackle the deadliest disease cancer. The inimitable topographical molecular architect encompassing this class of delivery system could allow the delivery of varying nature of anticancer bioactives viz lipophilic or hydrophilic drugs and macromolecules as proteins or RNA. The prospect of multifunctionality owing to multivalency, leads to decoration of their surface by different moieties for varying function to achieve a common goal and this could significantly enhance the efficacy of the transported bioactives. The conventional chemotherapy for cancer management exhibits a lack of selectivity and thus affecting healthy tissues. To realize selectivity, the dendrimers could be functionalized using moieties that would synergistically act to target the tumoral cells and release the payloads at the desired site. The approach of “cellular or secondary targeting” based on moieties that leads to ligand–receptor-

mediated endocytosis or of "tertiary targeting" based on moieties that recognize internal organelles or the use of stimuli-responsive moieties that are responsible for release of bioactives under specific internal or external stimuli, in combination onto a single dendritic structure synergistically act to achieve selective targeting [3,9,20].

3. Dendrimer-based multifunctional theranostics for cancer treatment

The unique features encompassing dendrimers enable their utilization for varied biomedical applications. Early prognosis of the fundamental molecular processes that cause cancer is anticipated from modern imaging technologies. However, the conventionally used contrast agents suffer with short imaging time, renal toxicity and lack of specificity. Dendrimers are now being explored as a platform for surface conjugation of various contrast agents including fluorescent dyes, iodinated contrast agents, and gadolinium (Gd) or radionuclide chelators and for entrapment, stabilization, or assembly of metal (e.g., Au), metal sulfides, and magnetic iron oxide NPs, leading to the formation of a range of contrast agents for different techniques including single mode fluorescence, computed tomography (CT), magnetic resonance (MR), positron emission tomography (PET), and single photon emission computed tomography (SPECT), and dual mode MR/CT, MR/fluorescence, PET/fluorescence, and SPECT/fluorescence. Further, the scope of incorporation of drug into ligand attached dendrimer-based contrast agents could pave the path to design dendrimer-based multifunctional theranostic agents for specific diagnosis and therapy of cancer [21-24].

Though, chemotherapy is currently considered to be the mainstream therapy for cancer management, however due to lack of selective targeting, it would affect healthy cells along with cancerous cells. Tumor physiology allows passive tumor targeting of drug-dendrimer conjugate by enhanced permeability and retention (EPR) effect [25]. In addition to this, molecularly active

targeted delivery of anticancer drugs on specific molecular target, could be achieved by surface functionalization of the dendrimer using various targeting moieties [26]. However, despite considerable efforts towards drug targeting, optimum outcome has not been observed and this may be due to a poor drug internalization and/or inefficient release of drug intracellularly. Efficient therapeutic strategy that could improve targeting and control drug release is currently the area of research in oncology domain. Thus, an efficient targeted drug delivery system should not only be able to achieve preferential accumulation and selective binding to the targeted cells but also been able to endorse cellular internalization, endosomal escape and control drug release [27, 28].

In view of the above, along with targeting ligand, dendritic system encompasses stimuli-responsive controlled release function that is responsive under specific internal or external stimuli. Alike the feasibility of passive targeting to tumor due to EPR effect, the release of the drug at target site could be controlled by exploiting the differential conditions existed at tumor microenvironment. Presence of biochemical gradient between tumor tissue and normal physiological tissue such as pH, redox potential, and enzymes can be exploited as internal stimuli for controlling the release of drugs. Among the external stimuli, use of feature that are responsive to temperature and light are attached onto dendrimers to create externally triggerable systems [29, 30].

Low pH at the extracellular space of solid tumors due to excessive accumulation of lactic acid and lower pH of some intracellular compartments such as the endosomes and lysosomes has driven interest in pH responsive assemblies for cancer therapy. Likewise, interest in redox sensitive carrier develops due to differences in the redox potential between extracellular space and the cytoplasm due to accumulation of reactive oxygen species (ROS) in cancer tissue.

Further, overexpression of various enzymes is observed in cancerous tissue due to augmented metabolic processes and thus could be used for designing enzyme responsive assemblies. Though, the utilization of external stimuli responsive assemblies like light and temperature responsive moieties are being researched for cancer drug delivery, however their utilization is associated with concerns regarding safety and penetration depth [31, 32].

The use of stimuli responsive smart linkers between drug and dendrimer is commonly utilized to control the release of free drug from drug-linker-dendrimer conjugate. The release of the drug in response to the stimuli is due to either reversible and irreversible transformations in the conjugate. However most of the stimuli leads to irreversible cleavage of the linkers. However, the stability and liability of the bond between the dendrimer, linker and drug under physiological conditions and in tumor tissue respectively governs the release behavior of the drug from drug dendrimer conjugate [33].

In addition to this, self-assembled dendritic systems that could disassembled in response to stimuli could also be employed to control dug release. Noncovalent interactions such as hydrogen bonding, π-π stacking and hydrophobic interactions are involved in self-assembly of dendritic systems [34]. The stability and nature of the aggregates are dependent on hydrophilic-hydrophobic proportion, and on external conditions such as temperature and concentration [35, 36].

Further, the class of self-immolative dendrimers is employed to control drug release which upon exposure to a specific trigger allow continuous degradation of their structure into small molecules [37]. In design, self-immolative dendrimers consists of a triggering unit connected to the branched skeleton composed of adaptor units. The adapter units are further attached with drugs as tail units. For controlled release of drugs, stimulation of trigger unit,

initiates rapid disassembly of the branched skeleton, with the subsequent release of all tail-units. “Dendritic amplification” or “Cascade-release” are the term used synonymously for the triggering response that leads to degradation of the conjugate [38-40]. Further, different self-immolative bonds can be used to adjust the degradation rate of the conjugate [41].

This review highlights various stimuli responsive dendritic assemblies utilized for the delivery of anticancer drugs. Different types of internal and external stimuli including acid, reduction potential, enzyme, temperature and light are discussed along with the respective responsive linkers that trigger the release in response to stimuli.

4. pH responsive dendrimers

The existence of pH gradient between tumor microenvironment (pH ~6.5) and normal tissues (pH ~7.4) act as a trigger for the controlled release of payloads from the drug delivery systems. An acid responsive functionality is anchored with such delivery devices, which remains stable in neutral and alkaline environment but gets degraded or hydrolyzed on exposure to acidic environment to release the drug. Further, some intracellular compartments, such as the endosomes and lysosomes have an acidic pH profile (4.5–6.5) that could trigger cytoplasmic drug release from acidic endo-lysosomal compartments [42].

Rapid proliferation effect in tumor tissues results in enhanced glycolysis instead of oxidative phosphorylation resulting in excessive accumulation of lactic acid and this will lead to slight decrease in pH of tumor extracellular environment. This decrease in pH is first observed by Warburg and is termed as Warburg effect [43, 44].

Presence of ionizable functional groups such as amine and carboxylic acid on surface or inner of dendrimer exhibit a pH-dependent release due to disruption of amphiphilicity of the

system. For example, low pH leads to protonation of tertiary amine which decreases the interior hydrophobicity of dendrimer and facilitate the release of payloads at the tumor site [45-47].

The pH gradient, also driven disassembly of assemble structure of amphiphilic dendrimers and thus aid in controlling release of payloads. Under specific pH conditions amphiphilic dendrimers assembled into micelles and these assembled structures tend to disassemble with altered conformation due to pH driven alteration in hydrophilic-lipophilic balance (HLB) [48, 49].

4.1 pH responsive linkers

The use of acid labile linkers could also be utilized in the construction of pH sensitive dendrimer. The acid labile linkers respond to variation in pH, they remain stable in neutral or alkaline pH, but degraded or hydrolyzed at acidic pH. Among pH-responsive linkers, the most frequently employed for anticancer drug delivery via dendrimer drug conjugate are hydrazone, acetal, and cis-acotinyl (**Table 1**) [42, 50].

4.1.1 Hydrazone linkage

Hydrazone linkages are most commonly used pH responsive linkage for designing dendrimer-prodrugs for cancer therapy. The hydrazone linkages hydrolyzed under acidic conditions and remain stable at neutral and alkaline pH [51]. The most common synthetic pathway for hydrazone is the condensation between hydrazines and ketones or aldehydes. Though hydrazones could also be synthesized by reaction between aryl halides and non-substituted hydrazones, and by reaction between aryl diazonium salts and beta-keto esters or acids (Japp-Klingemann reaction) [52].

A ketone or aldehyde group is required for hydrazone formation and thus hydrazine linkage is common with Doxorubicin (DOX)-dendrimer prodrug (**Figure 1**). Antitumor drugs,

without this functional group requires additional modification for conjugation via hydrazone bond. Drugs bearing hydroxyl group such as Paclitaxel (PTX), docetaxel (DTX) and cis-platin could be esterified with acid anhydride or carboxylic acid to obtain active keto sites for hydrazone formation.

Cheng et al, observed the pH-dependent release of DOX by formulating folic acid conjugated poly(ethylene glycol) (PEG)5000-PAMAM(G4) dendrimers using hydrazine linkers. *In vitro* release of DOX from PAMAM-DOX conjugate was evaluated at pH 4.5, 5.5 and 7.4, which was found to be 42, 28 and 8%, respectively. The results revealed the stability of hydrazine linker at pH 7.4 and lability at acidic pH [53]. In another report an amphiphilic linear-dendritic prodrug (mPEG-PAMAM-DOX) for the co-delivery of 10-hydroxy-camptothecin (HCPT) and DOX using acid-labile hydrazine linker was design and revealed acid responsive release behavior. It was observed that as the pH is decrease by 7.4 to 4.5 the release is increased from 5 to 60% revealing pH dependent cleavage of hydrazone linkage [54]. Likewise, She et al, designed mPEGylated peptide dendron-DOX (dendron-DOX) conjugate and demonstrated pH-dependent release of DOX using hydrazone linkage. The *in vitro* release revealed 20 and 80% drug release at pH 7.4 and 5, respectively [55]. Same research group also observed the release of DOX from galactose functionalized PEGylated dendrimer-DOX conjugates having hydrazone linkage. Due to cleavage of pH sensitive hydrazone linker, the release of DOX from the conjugates at pH 5 was much rapid than those at pH 7.4 [56].

Hydrazone linkage was successfully employed for conjugation of DOX to pH-sensitive drug-dendrimer conjugate-hybridized gold nanorods (PEG-DOX-PAMAM-AuNR). Drug release studies revealed that the release of DOX from the conjugate was negligible at pH 7.4, but was boosted considerably at weak acidic pH [57].

A biodegradable hyper-branched HPMA copolymer-DOX conjugate was synthesized with cathepsin B sensitive peptide GFLGK and the anticancer drug DOX was attached to the branched copolymer *via* a pH-responsive hydrazone bond. As compared to traditional copolymers, the biodegradable multiblock HPMA copolymer-drug conjugates resulted in enhanced anticancer efficacy with no obvious side effects [58].

4.1.2 Acetal linker

Chemically an acetal is an organic molecule having a central carbon atom attached to two oxygen atoms by single bond. For ketone derivatives, they are called ketals and are commonly used as protecting groups in organic synthesis and for the design of acid-sensitive linkages [59]. Acetal linkages can be formed by reaction between an aldehyde or ketone and alcohol [60] or between alcohol or phenols and vinyl ether, in the presence of acid catalysts [61]. Acetals are not stable to acidic environment and are very readily hydrolyzed back to the carbonyl and alcohol. However, there are only a few reports of application of acetals as pH-sensitive linkers for anticancer drug delivery using dendritic system.

The pH-responsive micelles based on PEO-dendritic polyester copolymer anchoring an anticancer drug, DOX by acid-labile acetal groups demonstrated the hydrolysis of acetal groups at acidic pH. The DOX was selectively released in tumor vicinity including endosomes and lysosomes [62].

4.1.3 Cis-acotinyl linker

The cis-aconityl, a derivate of natural aconitic acid is commonly used for controlling the release of amine group containing drugs. The interaction between cis-aconitic anhydride and an amine drug leads to a ring opening, which has a carboxylic functionality for conjugating to dendrimer. In mildly acidic conditions, the amide bond undergoes hydrolysis to release the drug.

In an interesting study Yabbarov et al, formulated a conjugate comprising rAFP3D (alpha-fetoprotein) acting as targeting ligand, PAMAM G2 dendrimer and DOX. The cis-aconityl linkage was used to conjugate DOX with PAMAM G2 dendrimer. The *in vitro* release study demonstrated that the release of DOX was found to be pH dependent with 8, 75 and 90% drug release at pH 7.4, 6.0 and 5.5, respectively [63]. Zhong and Da Rocha synthesized PEGylated G3 PAMAM-DOX conjugate by using an acid labile (cis-aconityl) and acid non-labile (succinic) linker. *In vitro* release studies conducted at pH 7.4 and 4.5 revealed 9 and 85% DOX release, respectively [64].

Similarly, Zhu et al, synthesized PEGylated PAMAM G4 dendrimers with different degrees of PEGylation and conjugated with variable amounts of DOX through cis-aconityl and succinic linker and term as PPCD and PPSD prodrugs, respectively. The *in vitro* release study showed negligible amounts of drug released from PPSD prodrug at varied pH values and pH dependent drug release from PPCD prodrug. The cytotoxicity study on murine B16 melanoma cells reveals significant toxicity by PPCD prodrug and negligible toxicity by PPSD prodrug [65].

4.1.4 Boronate ester linkers

Reaction between boronic acid and 1,2-diol or 1,3-diol in aqueous medium leads to the formation of boronate ester, a covalent ester bond. The bond is stable at pH higher than its pKa value but unstable at pH lower than its pKa value. Therefore, boronate ester can be used as pH sensitive linker to construct pH responsive assemblies [66, 67].

Boronate ester bond can be used to prepare bortezomib prodrugs. Catechol-modified PAMAM dendrimer was conjugated to an anticancer drug, Bortezomib via the boronate ester bond. The results revealed the drug release in acidic environment (pH 6.5) and no release at physiological pH [68].

4.1.5 N, O-chelate linker

A pH-responsive mPEGylated peptide dendrimer-linked diaminocyclohexyl platinum (II) (dendrimer-DACHPt) conjugate was prepared by Pan et. al. The DACHPt has a molecular structure, is and activity closely related to oxaliplatin. To achieve pH-sensitive DACHPt conjugation, the N,O-chelate was utilized to link the DACHPt to the dendrimers. The conjugate was pH-responsive and released drug significantly faster in acidic environments (pH 5.0) than pH 7.4. The result revealed that the conjugates suppressed tumor growth better than clinical oxaliplatin without inducing toxicity in an SKOV-3 human ovarian cancer xenograft [69].

5. Redox-responsive dendrimers

Control over release of drug in response to difference in the reduction potential between tumors and normal tissue is frequently employed strategy in cancer therapy. There is highly regulated redox status inside the normal cell balancing the reduced and oxidized species. This balance gets disturbed in cancerous cells, which leads to accumulation of ROS and results in oxidative stress. To overcome oxidative stress, cells recruit ROS scavengers such as glutathione (GSH) and vitamins C and E. The significant difference (about 4-fold) in GSH concentration intracellularly (2-10×10^{-3} M) and extracellularly (2-20 μM) in cancerous tissue have made GSH responsive assemblies most explored for reductive responsive drug delivery systems [70-72]. Further, a specific reducing enzyme, gamma-interferon-inducible lysosomal thiol reductase (GILT) modulates the redox potential of endosomal compartment in the co-presence of a reducing agent such as cysteine [73-75].

The frequently used redox-reponsive linker for dendrimer drug conjugate is disulphide linker. The elevated GSH mediates disulfide bond cleavage reactions via reduction or dithiol-disulfide exchange process (**Figure 2**). Besides disulfide bonds, diselenide or ditellurium bonds

are also used as redox responsive linkers. The diselenide bond is more sensitive than disulphide bond towards stimuli as the cleavage energy of diselenide bonds is lesser as compared to disulfide bonds [76-78].

A novel stimulus responsive conjugate of dendrimer and gold nanoparticle (GNP) was developed for the delivery thiolated anticancer drugs by Wang et al. Dendrimer-encapsulated gold nanoparticles (DEGNPs) were synthesized and thiolated anticancer drugs are attached through the Au-S linkage. The conjugate exhibited an "Off-On" release behavior in the presence of thiol-reducing agents such as glutathione and dithiothreitol. The developed conjugate showed much reduced cytotoxicity as compared to the free anticancer compounds [79].

A new class of disulfide cross-linked G2 PAMAM dendrimers was prepared as non-viral gene carrier to enhance transfection efficacy and to reduce cytotoxicity. Disulfide containing linker 3,3′-dithiodipropionicacid-di(N-succinimidyl ester), (DSP) was used to cross-link G2 PAMAM dendrimers to form supra-molecular structures (G2DSPs). The cross-linked conjugate was degraded due to disulfide bond reduction after gene transfection and this regulated the release of DNA in a controlled manner [80].

In a recent study a redox responsive peptide conjugated tumor targeted nano vehicle (PSPGP) composed of branched PEG with G2 dendrimers was synthesized for co-delivery of PTX and siTR3 for treatment of pancreatic cancer. The assembly was conjugated with PTP (plectin-1 targeted peptide, NH_2-KTLLPTP-COOH), a biomarker for pancreatic cancer. Redox-responsive disulfide bonds were used to link the PTX and siTR3 to the conjugate. The complex showed inhibition in tumor growth and promoted cancer cell apoptosis [81].

Lim et al, synthesized 3 conjugates of PTX with PEGylated triazine dendrimer. The dendrimer construct 1 includes an ester linker, whereas dendrimer construct 2 and 3 include a

disulfide linker. Cytotoxicity studies using an MTT-based assay and PC-3 cells revealed IC_{50} values of 4.5 and 29 nM for free PTX and construct 1, respectively and increased in construct 2 and 3 from 74 to 26 nM and 13nM in the presence of 1 mM glutathione and 1 mM dithiothreitol, respectively [82].

Reduction-responsive amphiphilic dendritic copolymer (TPP-S-S-G3) with disulfide-linkages between dendrimer (PEG-G3-OH) and porphyrin (TPP, photosensitizers) for the combined chemotherapy and photodynamic therapy (PDT) was developed. The copolymer self-assembled into micelles in aqueous solution. The results showed fast uptake and release of DOX-loaded TPP-S-S-G3 micelles by MCF-7 cells [83]. Nguyen et al, studied Heparin (Hep) conjugated to PAMAM G3.5 (P) via redox-sensitive disulfide bond (P-SS-Hep). The dendrimer complexes were found to promote redox-sensitive drug release intracellularly. In the cancer cells the disulfide linkage cleaved and enabled the release of drug. Hence, providing evidence of potential of redox sensitive nanocarriers in cancer chemotherapy [84].

Dual responsive PAMAM dendrimers that responded to variation in reduction potential and pH have been used for the delivery of DOX. The redox-responsive functionality is imparted using disulfide linkage between PAMAM dendrimers and PEG with DOX loaded into the hydrophobic core of the conjugates. The release study demonstrated redox and acid trigger release behavior of DOX [85]. For tumor-targeted drug delivery an asymmetric bow-tie PAMAM dendrimer (ABTD) scaffold has been developed using disulfide unit as self-immolative linker. The results revealed a remarkable selectivity of ABTD scaffold to cancer cells as compared to human normal cells and demonstrated reduction responsive release behavior [86].

A GSH-triggered self-immolative dendritic prodrug has been designed for cancer therapy. The assembly comprised an anticancer drug Camptothecin (CPT), a reduction cleavable

linker (2,4-dinitrobenzenesulfonyl, DNS) and a near infrared (NIR) fluorescent dye (dicyanomethylene-4H-pyran, DCM). Cleavage of the DNS linker in the presence of GSH released the drug and activated NIR fluorophore, which could aid to track the released drug [87].

To develop highly efficient and safe gadolinium (Gd)-based MRI contrast agents with minimum bio-accumulation and least detrimental effect on the body, Guo et.al, develop biodegradable Gd-based polymeric contrast agents with a biocleavable disulfide linker. Biodegradable poly[N-(1,3-dihydroxyprpyl) methacrylamide] copolymers (pDHPMA) were synthesized and small molecular Gd-chelate (Gd-DOTA) was conjugated onto the copolymer backbone through a sulfide bond or a GSH-sensitive cleavable disulfide bond to produce two novel Gd-mCAs (pDHPMA-Cy5.5-DOTA-Gd or pDHPMA-Cy5.5-SS-DOTA-Gd) for tumor diagnosis. The developed contrast agents demonstrated enhanced relaxation efficiency, improved pharmacokinetics and better passive targeting through EPR effect as compared to Gd-diethylenetriamine pentaacetic acid (DTPA-Gd) [88].

6. Enzyme-responsive dendrimers

Changes in the level and activity of various enzymes are observed in cancer etiology. As, cellular metabolic processes are augmented in cancer tissue, the enzymes that regulate these processes are commonly overexpressed. This dysregulation of their expression is considered as characteristic feature of the cancer and is utilized as a tool in diagnostics. Along with diagnostics, such dysregulation is utilized in managing the disease condition by programming the drug delivery system for active targeting and to control the release of drugs. The on-demand drug release, governed by enzyme is designed by integrating specific linkers that can be recognized and degraded by the enzymes overexpressed in the extracellular or intracellular environment of the tumor [89, 90]. A variety of enzymes are found to be upregulated in tumor tissues including

cathepsins, matrix metalloproteases (MMPs), hyaluronidase, azoreductase, phospholipase and many more [91].

Further, the advent of enzyme responsive self-immolative dendrimers as molecular amplifiers has translated the release of drug on enzymatic stimulation. Incorporation of drug molecules as the tail units and an enzyme substrate as the trigger in self-immolative dendrimers, generated a prodrug unit that was triggered upon a single enzymatic cleavage. The enzymatic trigger commonly utilized for the same is 38C2 antibody, penicillin-G-amidase and β-galactosidase [92, 93].

Cathepsins, a group of proteolytic enzymes predominantly located in endo/lysosomal vesicles, are involved in the degradation of extracellular matrixes (ECM) of the tumor tissue and thus contributing to infiltration of the tumor cell. Out of various cathepsins, cathepsin B is one of most explored lysosomal proteases due to its high expression in various types of cancers including prostate, breast, lung, brain, endometrium and colorectum. Invasive and metastatic cancers are the results of abnormal regulation of cathepsins [94, 95].

Lee et al, synthesized dendrimer-methoxy PEG (MPEG)-DOX conjugates using a cathepsin B-cleavable peptide, glycyl phenylalanyl leucyl glycine tetra-peptide (Gly-Phe-Leu-Gly) for anticancer drug targeting (**Figure 3**). The results revealed improved anticancer activity in an *in vivo* CT26 tumor xenograft model *i.e.* the volume of the CT26 tumor xenograft was significantly inhibited [96].

Cathepsin B-cleavable peptide (Gly-Phe-Leu-Gly) was successfully used to develop a novel enzyme-responsive PEGylated lysine peptide dendrimer-gemcitabine (GEM) conjugate (Dendrimer-GEM) based nanoparticle. The results indicated suppressed relative tumor volumes

(86.17±38.27%) and a 2-fold higher value of tumor growth inhibition (~90%) than GEM, establishing enhanced antitumor efficacy without obvious systemic toxicity [97].

In another study cathepsin B-cleavable peptide was utilized by Zhang and coworkers to develop mPEGylated peptide dendrimer-DOX (dendrimer-DOX) conjugate-based nanoparticles, which demonstrated significantly high antitumor activity and substantially reduced DOX-related toxicities [98]. Similar peptide along with a pH-sensitive hydrazone bond was exploited by Chen et al, for the preparation of a novel pH/enzyme sensitive dendritic polymer-DOX conjugate for cancer treatment. The result revealed high accumulation of DOX into tumors due to prolonged blood circulation time. *In vivo* studies revealed better antitumor efficacy of the conjugate in comparison with free DOX [99].

Wang et al, designed an enzyme-stimuli nanogel based on G4 PAMAM dendrimers using elastase cleavable bond (Ac-arg-ala-ala-asp-D-tyr-cys-NH_2) (RAADyC). Neutrophil elastase (NE) is detected in different types of cancers, and its concentration is associated with the cancer stage, grade, and the survival [100].

Azoreductase, an enzyme over-expressed in hepatocellular carcinoma cells, can work as a trigger to induce drug release. Medina et al, synthesized a series of aromatic azo-linkers (L1-L4), which were used to conjugate DOX to G5 PAMAM dendrimers. To study the effect of electronegativity on susceptibility to cleavage by azoreductase enzymes, these linkers are incorporated with electron-donating oxygen (O) or nitrogen (N) groups. Results revealed the release of 4-8, 17, 60, and 100% of the conjugated DOX molecule from dendrimers having linkers L1 to L4, respectively. Increase in electronegativity increases susceptibility to cleavage by azoreductase enzymes [101].

Phospholipase C (PLC) enzyme, an important regulator of membrane phospholipid metabolism is found to be overexpressed in many cancers and participates in cancer cell progression and differentiation [102,103]. Zhang et al, synthesized enzyme-responsive phosphoramidate (PAD) dendrimers for delivery of DOX. The dendrimers were degradable in the presence of PLC but found to be stable in phosphate buffer saline (PBS). The phosphite ester bonds in PAD dendrimers is degraded by PLC. The results revealed improved therapeutic efficacy of the conjugate with reduced toxicity in athymic nude mice bearing xenografts of MCF-7/ADR breast cancer [104].

A dendritic prodrug with an anticancer agent camptothecin (CPT) and a trigger that allowed activation by penicillin-G-amidase was designed and synthesized. Cell-growth inhibition assays demonstrated that the toxicity of the dendritic prodrug was found to be dependent upon incubation with penicillin-G-amidase [105]. Shamis et al, prepared a self-immolative assembly for synergistic combinational therapy in cancer utilizing DOX and CPT as tail units and a catalytic antibody 38C2 cleavable retro-aldol retro-Michael focal trigger [106].

In an effort to improve therapeutic index of an anti-cancer drug, gemcitabine (GEM), a stimuli-responsive dendritic polyHPMA copolymer was designed and synthesized GEM (Dendritic polyHPMA-GEM) prodrug via one-pot synthesis of RAFT polymerization by Dai and coworkers. GEM was conjugated onto the dendritic polymeric carrier via an enzyme-responsive linker glycyl-phenylalanylleucyl-glycine tetra-peptide (GFLG), which was found to be stable in blood circulation system and degraded in the presence of Cathepsin B only. The results revealed that the designed stimulus-responsive dendritic copolymer-GEM prodrug may a safe, effective and enzyme-responsive anticancer agent [107].

Polymer-drug conjugates has significantly improved the anti-tumor efficacy of chemotherapeutic drugs and alleviated their side effects. In this regard a biodegradable diblock N-(1,3-dihydroxypropan-2-yl) methacrylamide (DHPMA) copolymer-DOX conjugate (a self-aggregation-induced nanoprodrug) via one-pot of RAFT polymerization and conjugate chemistry was developed. Notably, the nanoprodrug had a significantly prolonged blood circulation time with an elimination half time of 9.8 h. It displayed high accumulation within tumors, and improved e *in vivo* therapeutic efficacy against 4T1 xenograft tumors compared to free DOX. The authors demonstrated that the diblock pDHPMA-DOX nanoprodrug with a controlled molecular structure exhibited an enhanced antitumor efficacy against 4T1 breast tumors through the inhibition of cell proliferation and antiangiogenic effects and alleviated side effects, showing a great potential as an efficient and safe anticancer agent [108].

7. Temperature-responsive dendrimers

Among external stimuli, temperature trigger drug release has shown significant potential. However, the use of temperature as a trigger requires external heating methodology that can heat the tumor area locally and thus respond to temperature variation [109]. Modification of dendrimer surfaces with oligo- and poly-ethylene oxide-based functionality endow them with temperature-sensitive characteristics [110].

There is an inverse relationship between aqueous solubility and temperature for temperature sensitivity functionalities. As temperature is increased the degree of hydrogen bonding between the temperature sensitive moieties and water decreases, and this will leads to phase separation. Lower critical solution temperature (LCST) or the cloud point is the phrase used demark such phase transition and is specific for a moiety [111]. Most commonly used thermo-responsive material includes PEG and poly(N-isopropylacrylamide) (pNIPAM). These

functional groups become hydrated due to hydrogen bonding with water and the application of temperature trigger breakdown of these weak interactions causing them to lose its hydrophilicity [112,113].

Thermosensitive pNIPAM polymer-conjugated PAMAM dendrimer has efficiently delivered Malloapelta B (Mall B) against HepG2 cancer cell proliferation. The conjugate showed high encapsulation of Mall B and demonstrated slow controlled release, which significantly inhibited HepG2 cancer cell proliferation [114]. Wu et al, synthesized G4 thermosensitive dendrimers based on oligo (ethylene glycol) (OEG) conjugated with an antitumor agent, GEM. The prepared dendrimers were compared with that of GEM-conjugated PAMAM dendrimers. The GEM-OEG based dendrimers exhibited thermal responsive release behavior and better tumor accumulation and penetration than the GEM-conjugated PAMAM [115].

A temperature responsive dendrimer conjugate was prepared for gene silencing through intracellular small interfering RNA (siRNA) release. The pNIPAM and phenylboronic acid were conjugated with PAMAM dendrimer for the design temperature responsive system. The phenylboronic acid improves the stability and cellular uptake while pNIPAM is responsible for temperature responsive behaviors at lower critical solution temperature. The results revealed that gene silencing efficacy was significantly increased by cool treatment after its cellular uptake with minimal toxicity [116].

Though, temperature-sensitive materials for dendrimer drug conjugate is numerous, a few are potentially utilized for temperature-responsive drug release. This is probably due to difficulty in controlling the release of the drug during phase transition and the safety concerns of the temperature-sensitive polymers above LCST for *in vivo* applications. Further, it is very difficult to heat localized tissues without hurting normal tissues.

8. Light-responsive dendrimers

As external stimuli, light is most explored due to some obvious advantages such as non-invasiveness and prospect of temporal and spatial accuracy. The principle governing the release of drug from dendrimers using light as a stimulus is based on- (i) the absorption of light by photosensitive ligands that would trigger reversible physical changes (e.g., trans-cis isomerization) and cause release of the encapsulated drugs and (ii) the absorption of light by photosensitive ligands causes irreversible cleavage reaction. The most common photosensitive ligands for the former are azobenzene derivatives and for later are o-nitrobenzyl ether (or ester) derivatives grafted on the surface of dendrimers [117].

The commonly used light triggers includes ultraviolet (UV) (200-400 nm), visible (400-700 nm) or near-infrared (NIR) (700-1000 nm) light. However, UV and visible light usage gives poor tissue penetration as well as leads to phototoxicity. NIR light irradiation has deeper tissue penetration with the penetration depth of up to 2 cm with less phototoxicity and thus preferred. Nevertheless, NIR light has inherent low energy and due to this two-photon excitation technique would be considered ideal for photobiological applications using NIR light irradiation or the application of upconversion nanoparticles, which can convert adsorbed NIR light to UV irradiation (**Figure 4**) [118-120].

Choi et al, designed folic acid conjugated G5 PAMAM dendrimer and photocaged DOX using the photocleavable group ortho-nitrobenzyl (ONB) (**Figure 5**). The *in vitro* cytotoxicity studies using KB cell-based assay revealed release of DOX and cytotoxicity on exposure to UV light [121]. Similarly, in another study same group of researchers designed targeted PAMAM dendrimer for the delivery of methotrexate (MTX). The *in vitro* cytotoxicity study using KB cell-based assay demonstrated MTX release through a light-controlled mechanism following

exposure to UV light [122]. Sun et al, designed DOX loaded Janus-type dendritic structure by linking a hydrophobic dendron (diazonaphthoquinone (DNQ)-decorated G3 PAMAM) and a hydrophilic dendron (lactose (Lac)-decorated Gm PAMAM dendrons). The DNQ, undergoes a Wolff rearrangement to form a ketene, on exposure to light. These Janus dendritic structure gets self-assembled into micelle in aqueous solution and gets disassemble on exposure to NIR light. The results presented a photo-triggered cytotoxicity and revealed doubling of DOX release on irradiation to NIR [123].

Coumarin, a natural dye, with high two-photon cross sections is utilized as photocages for the light responsive release of chemotherapeutic drugs [124]. Wang et al, synthesized a light responsive construct for the co-delivery of 5-Flurouracil (5-Fu) TRAIL plasmid for cancer therapy. The anticancer moieties were loaded on amphiphilic G1 dendrimer-coumarin conjugate (G1-CM). Coumarin acts as photoresponsive group and on exposure to light leads to degradation of the assembled structure and exhibits a light-responsive drug release profile [125].

Thioacetal ortho-nitrobenzaldehyde TNB(OH) photolinker was utilized for the construction of TNB-caged DOX conjugates. The constructed caged conjugates are then integrated with 2 folic acid functionalized nano-assemblies. First is, G5 PAMAM dendrimer and second is upconversion nanocrystal (UCN) conjugate with protoporphyrin IX (PPIX) as cytotoxic photosensitizer. Cellular toxicity studies in KB carcinoma cells revealed that each nano-assembly exhibit cytotoxicity on exposure to UV or NIR (980 nm) [126]. However, despite various obvious benefits of light as a stimulus for drug release, its application is limited in the treatment of solid tumor due to the ambiguity regarding penetration depth, irradiation time and effective area.

9. Smart dendrimers in gene delivery

An alternative strategy to traditional radiotherapy and chemotherapy, gene therapy is now recognized as a potential therapeutic modality for cancer treatment. Gene therapy has been extensively explored for the management of cancer, as approximately 65% of the clinical trials in gene therapy have been designed at the treatment of various types of cancers [127]. To realize gene transfer complex cellular and tissue barriers must be overcome without disrupting vital regulatory mechanisms to deliver the tailored therapeutic gene for augmentation or suppression or repair, using a vehicle called vector [128].

In addition to carrier of chemotherapeutic agent and contrast agent in molecular imaging for cancer treatment and prognosis, dendrimers are also considered as non-viral vector for gene therapy. In contrast to viral vectors, dendrimers as a non-viral vector offer distinct advantage including target-cell specificity and resistance to repeated administration. Further, the biodegradable dendrimers are preferred for gene delivery as compared to the non-degradable dendritic vectors, due to their reduced toxicity and degradability. The PAMAM dendrimers, dendritic polyglycerols and peptide dendritic polymers are the commonly investigated vectors for gene therapy [129, 130].

Alternatives to viral-mediated gene delivery, dendrimers are now being largely investigated as an effective non-viral mediated gene delivery system. Though, viral vectors have high transfection efficacy but are accompanied by high immunogenicity, cytotoxicity and production problems. Owing to possibility of multifunctionality, dendrimers are perceived as non-viral vector that can overcome these limitations [131]. However, cationic dendrimers is associated with serious toxicity and thus a key challenge in clinical gene therapy is to prepare dendritic vector with high transfection efficacy and low toxicity. Fluorinated dendrimer, a new class of non-viral gene carriers exhibits interesting physicochemical properties, with efficient

cellular internalization and less toxicity [132,133]. A structure-activity relationships (SAR) study for DNA and siRNA delivery based on different dendrimer generations and fluorination degrees reveals that fluorination significantly improves the transfection efficacy of G4-G7 PAMAM dendrimers. Fluorination on G5 dendrimer yields the most efficient polymers in gene delivery, and the transfection efficacy of fluorinated dendrimers depends on fluorination degree. All the fluorinated dendrimers cause minimal toxicity on the transfected cells at their optimal transfection conditions [134].

A series of fluorodendrimers was synthesized, by reacting PAMAM dendrimers with heptafluorobutyric anhydride, as non-viral gene vectors. The synthesized conjugate self-assembled to form uniform polyplexes with promising properties at a low nitrogen-to-phosphorus ratio and have low charge densities and relatively weak DNA associations. Uniform polyplexes ensures reproducible gene transfection. A low charge density indicates low cytotoxicity and weak DNA association, which is beneficial for efficient DNA unpacking in the cytoplasm [135].

The interaction of G5 PAMAM dendrimers with perfluoro acid anhydrides resulted in the development of fluorinated dendrimers with high transfection efficacy and low toxicity. The study revealed that fluorination of the dendrimers improved the transfer across cell as well as the endosome/lysosome membrane facilitating endosomal escape. Further, this class of dendrimer has shown to form polyplexes with genes at low nitrogen to phosphorus (N/P) ratios to minimize the toxicity on the transfected cells [136].

A stimulus-responsive fluorinated bola-amphiphilic dendrimer bearing ROS-sensitive thioacetal in the hydrophobic core and positively charged PAMAM dendrimer at the terminals was synthesized for the delivery of siRNA in cancer cells. The conjugate combine the

advantageous delivery features of both lipid and dendrimer as a non-viral vector. The result revealed that the conjugate capable of interacting and compacting the negatively charged siRNA into nanoparticles to protect the siRNA and promote cellular uptake [137].

A heptafluorobutyric acid modified G4PAMAM dendrimer (G4-F735) has been used as a nonviral vector to deliver plasmid encoding TNF-related apoptosis-inducing ligand (pTRAIL) gene for cancer treatment to achieve both excellent transfection efficacy and low toxicity. The results revealed much higher TRAIL gene transfection efficacy than a series of transfection reagents including poly(ethylene imine) (PEI), SuperFect and Lipofectamine 2000 and exhibited minimal toxicity *in vitro* [138].

For the investigation of fluorous effect on transfection efficacy and cytotoxicity, Wang and Cheng synthesized a series of fluorobenzoic acid (FBA)-modified dendrimers as non-viral gene vectors. The results demonstrated that the transfection efficacy increases with increasing number of fluorine atoms on the aromatic rings. The modified dendrimers were found to be superior as compared to the polymer-based and lipid-based commercial reagents such as SuperFect, PolyFect, and Lipofectamine 2000, respectively. Fluorination on the aromatic rings significantly improves the transfection efficacy of benzoic acid-modified dendrimers [139].

In a study fluorodendrimer was prepared by reacting G2 PAMAM dendrimer with heptafluorobutyric anhydride for the co-delivery of fluorinated anticancer drugs (sorafenib or 5-Fu) and therapeutic genes (TRAIL plasmid) in synergistic cancer therapy. The results revealed high drug loading and gene transfection efficacy with minimal toxicity [140].

10. Conclusion

The synergy that exists between experimental and theoretical studies opens new avenues for the use of dendrimers as versatile drug delivery systems. The possibility of diverse functionalization

on dendritic structure paves the path for delivery of drugs in spatial-, temporal- and dosage-controlled fashions for cancer therapy. The use of stimuli responsive smart linkers facilitates the delivery of payloads in a controlled manner on specific triggers. The incorporation of pH and redox responsive systems into dendrimers, has attracted significant interest. Various functional groups have been utilized in dendritic assemblies such that a pH sensitive linker would provide stability to the assembled nanostructure stable at neutral pH 7.4, but would respond to a lower pH. The use of pH-, redox-, enzyme-, thermal- and light-responsive ligands potentiate the target functionalized dendrimers in delivering anticancer bioactives in an efficient and safer manner.

Declaration of interests

None

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Table legend:

Table 1. Acid-responsive chemical bonds and corresponding degradation products under acidic environment [Modified and reproduced with permission from Ref 42]

Figure legends:

Fig 1. Dendrimer-doxorubicin conjugate via hydrazone linkage [Modified and reproduced, with permission from Ref 32]

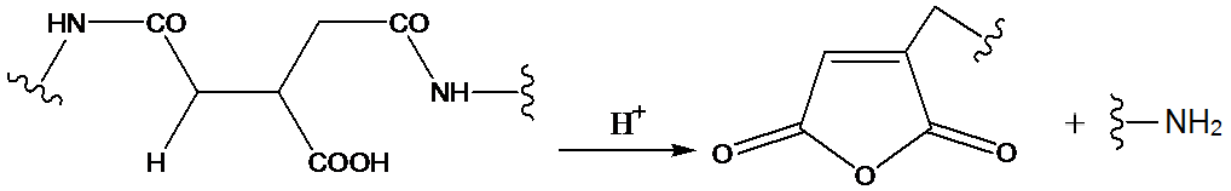
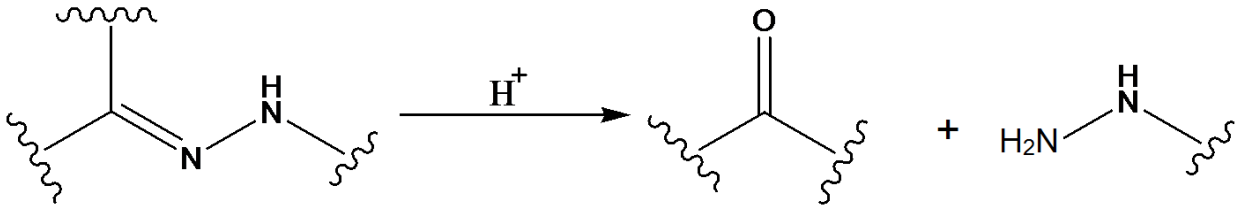
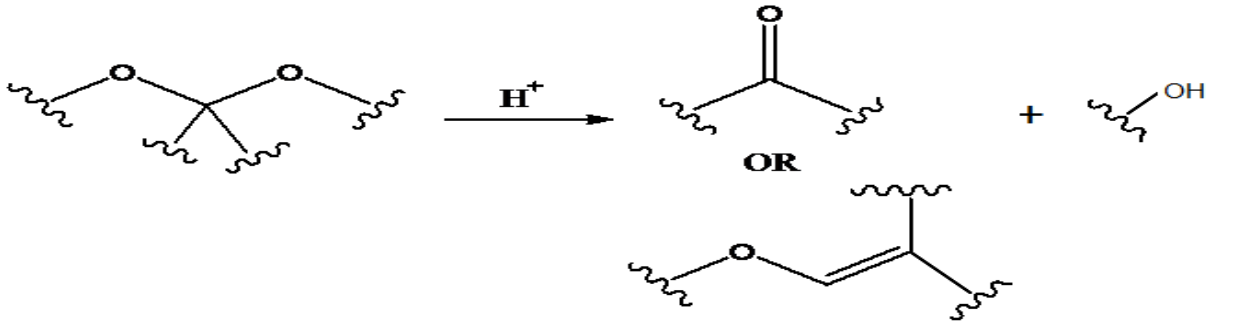
Fig 2. Cleavage of disulfide bond between dendrimer–drug conjugates via GSH [Modified and reproduced, with permission from Ref 32]

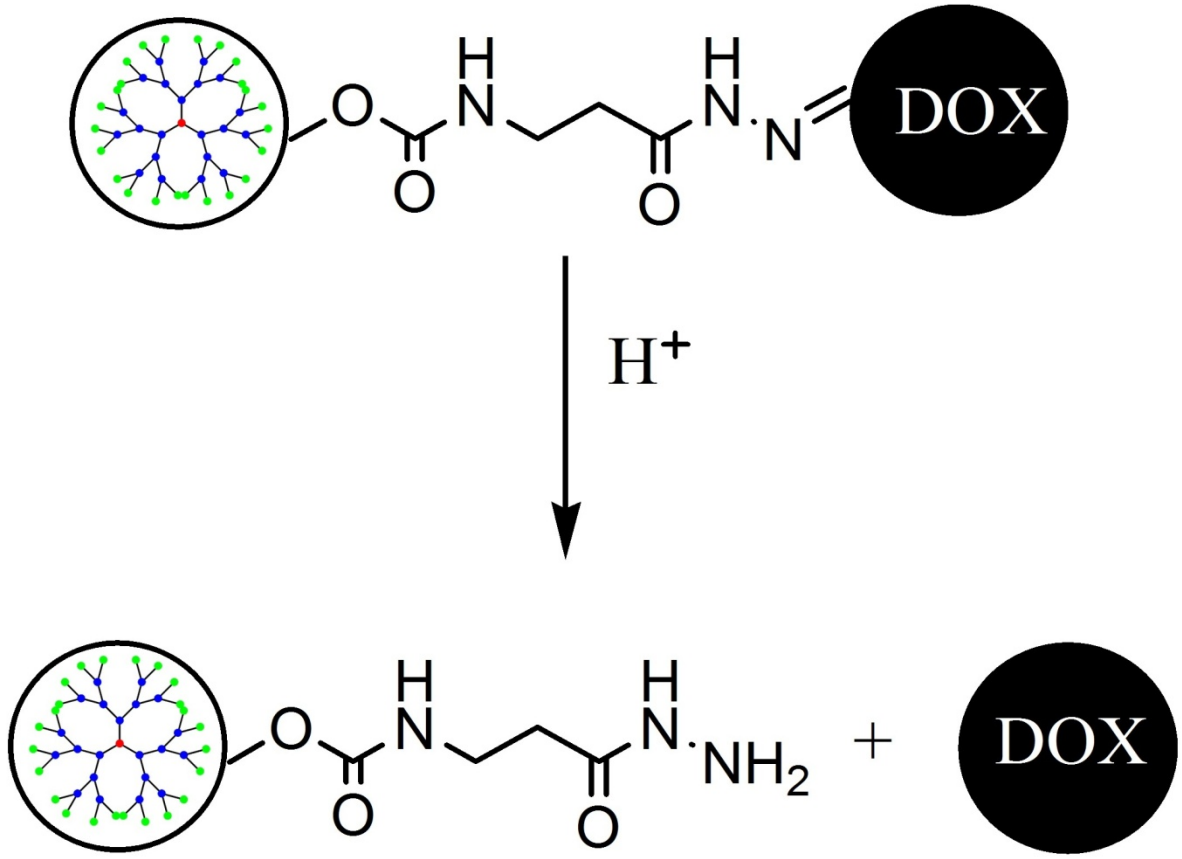
Fig 3. Cleavage of glycyl phenylalanyl leucyl glycine tetra-peptide (GFLG) between dendrimer-doxorubicin conjugates by Cathepsin-B [Modified and reproduced, with permission from Ref 32]

Fig 4. Photo-cleavable groups: (a) Ortho-nitrobenzyl (ONB) group and (b) Coumarin [Modified and adopted from Ref 118].

Fig 5. Cleavage of ortho-nitrobenzyl (ONB) linker in dendrimer-doxorubicin conjugate upon UV light irradiation [Modified and reproduced, with permission from Ref 32]

Table 1. Acid-responsive chemical bonds and corresponding degradation products under acidic environment [Modified and reproduced with permission from Ref 42]

Acid-responsive chemical bond	Structure	Degradation products	Reference
Cis-Aconityl			[42]
Hydrazone			
Acetal			



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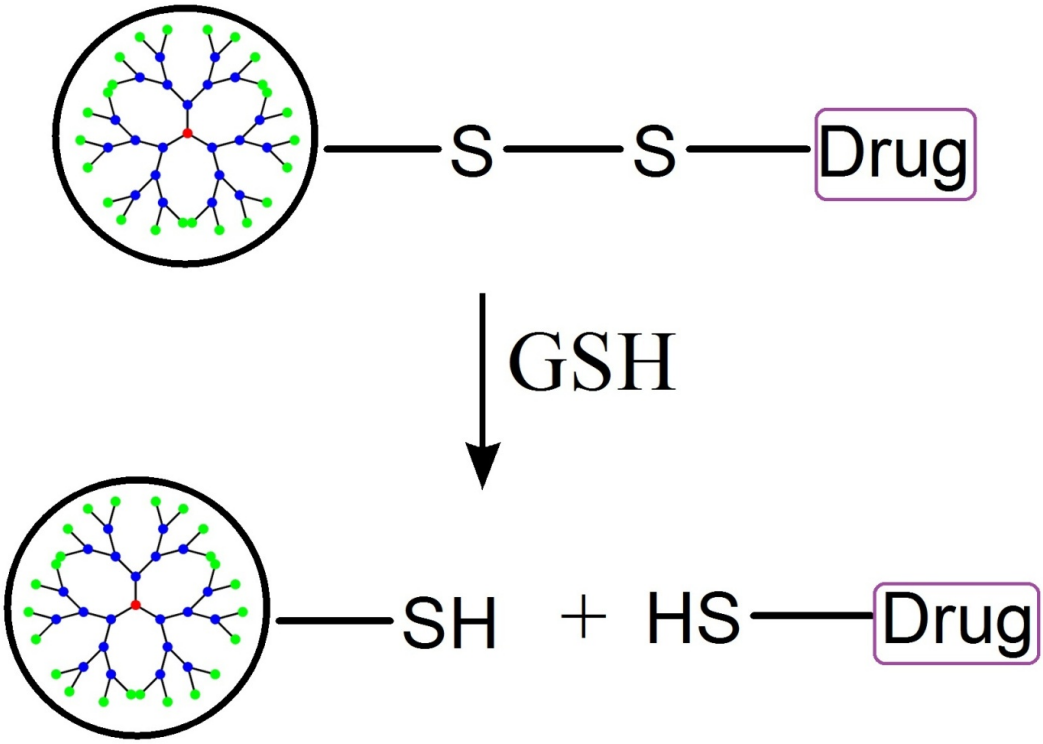
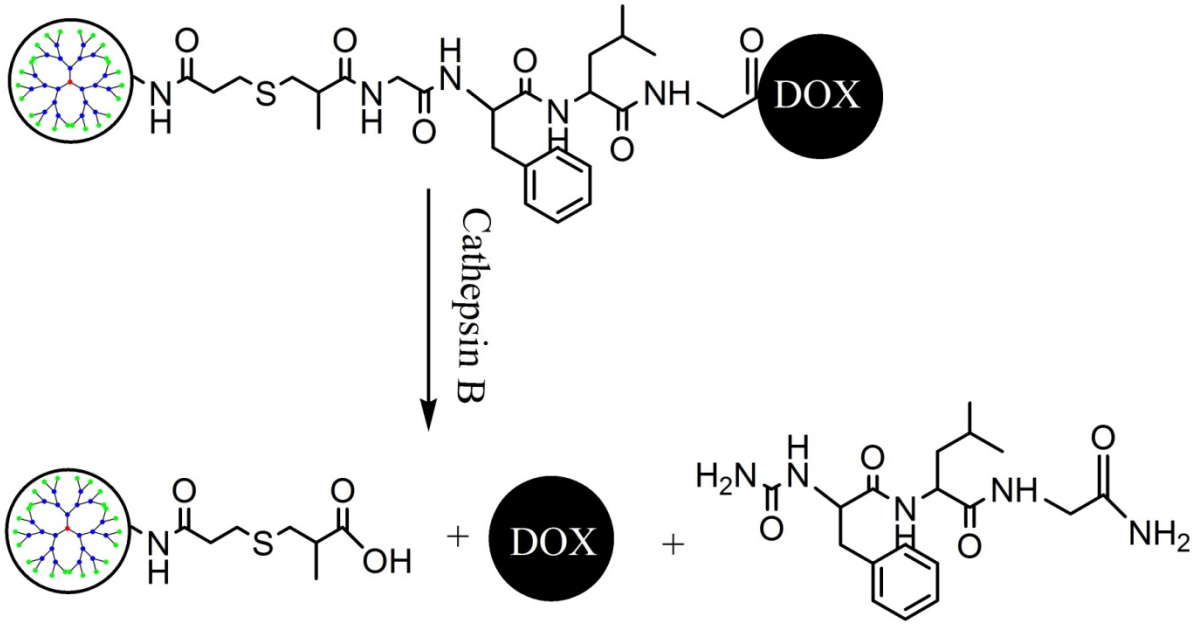


Fig 2. Cleavage of disulfide bond between dendrimer–drug conjugates via GSH [Modified and reproduced, with permission from Ref 32]



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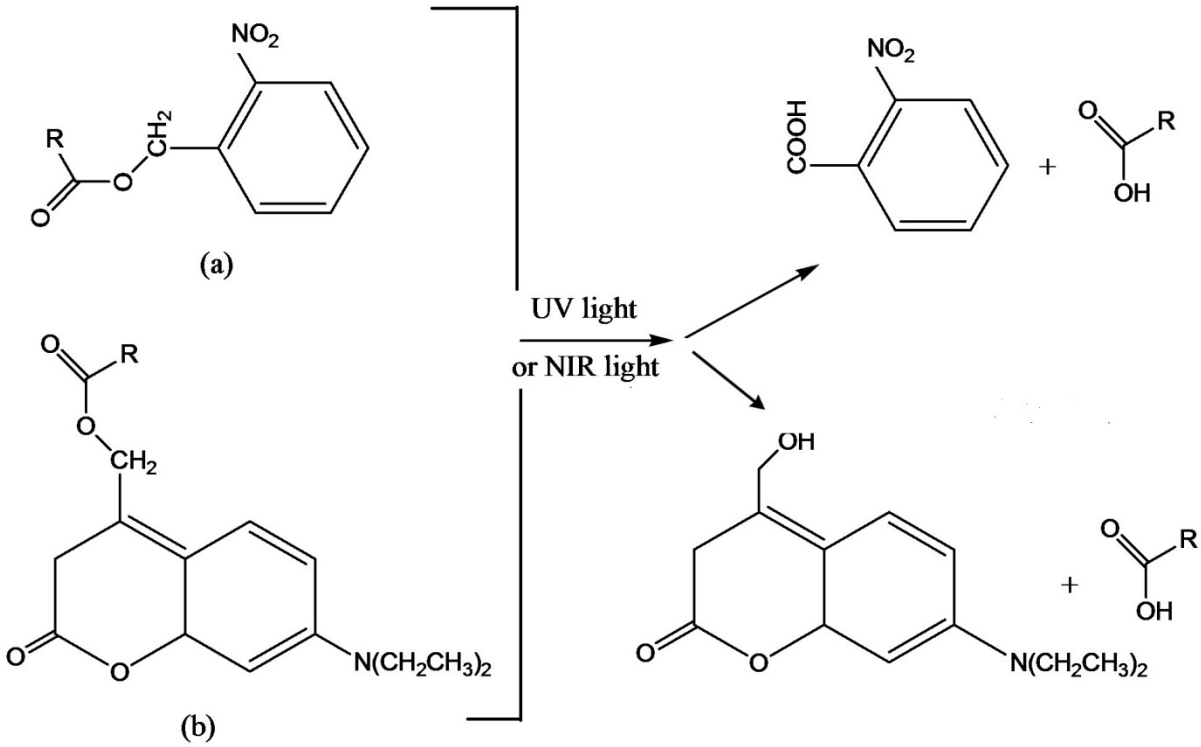

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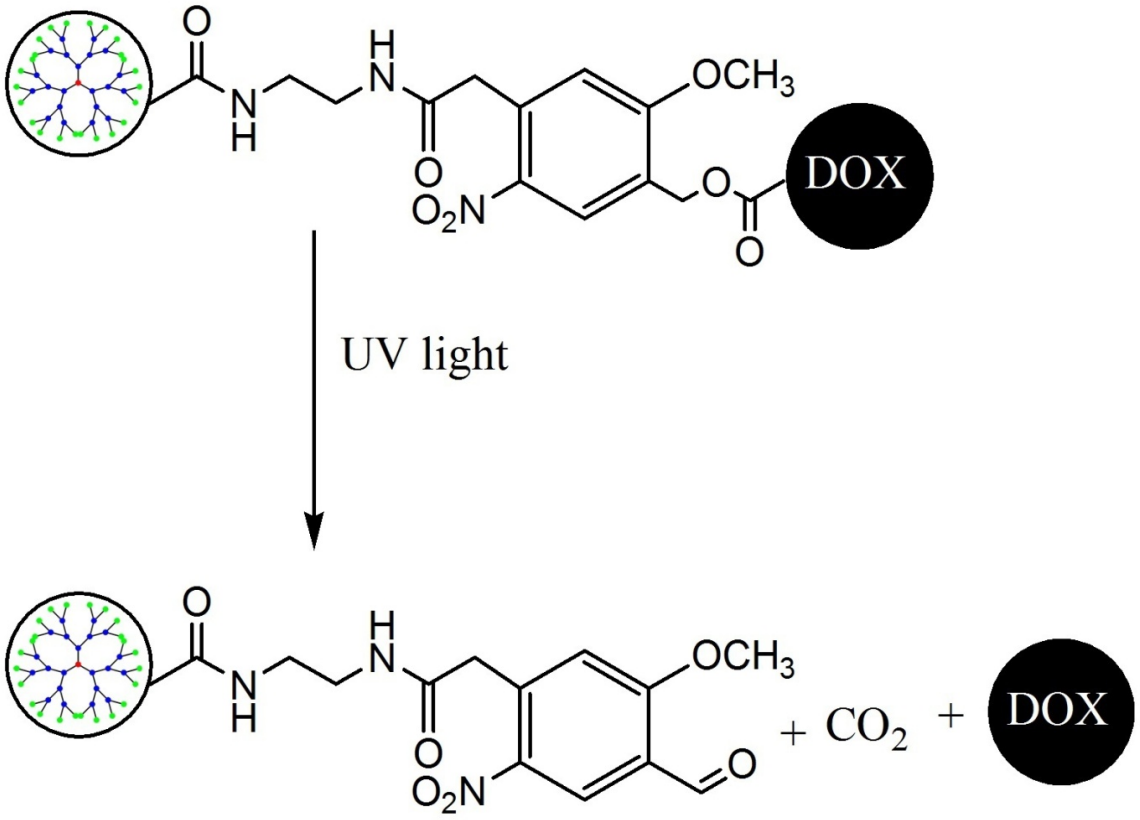

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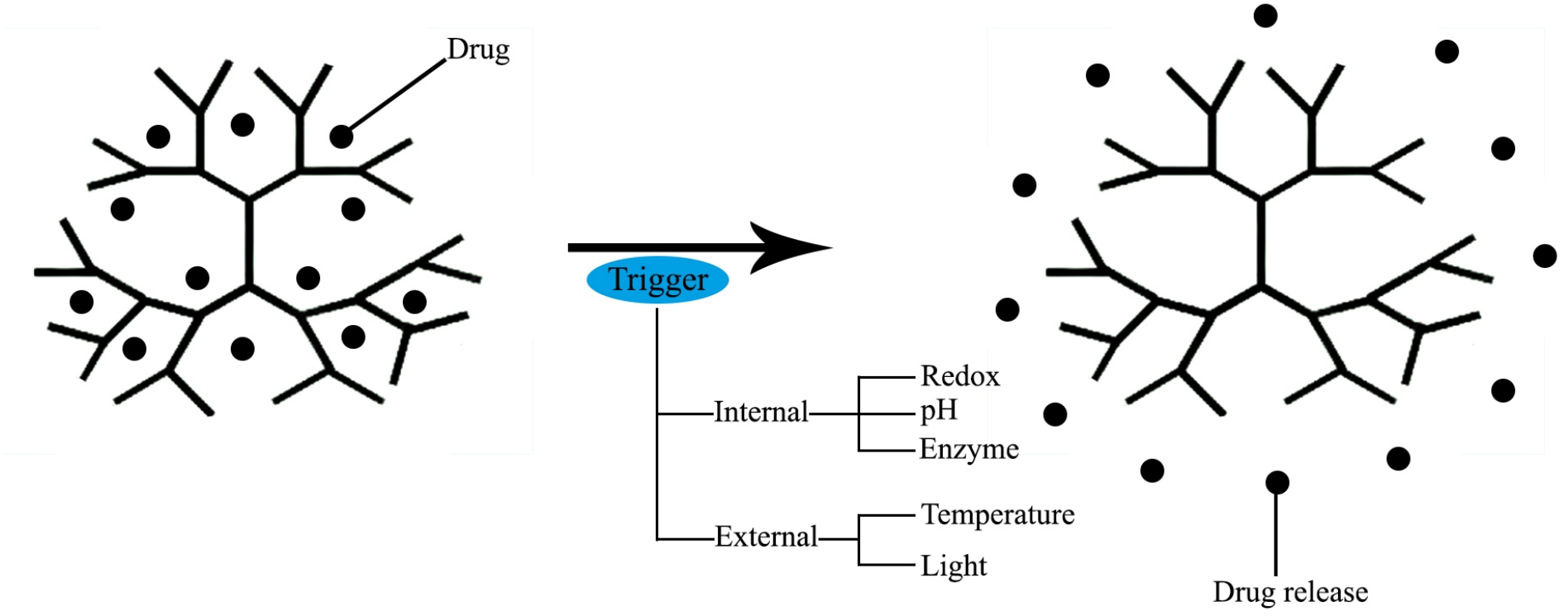

Stimuli triggered drug release from dendrimers

Smart dendrimers: Synergizing the targeting of anticancer bioactives

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Abstract

Optimization of biological performance of a carrier in cancer drug delivery depends on the targeting potential of the delivery system and its ability to control the drug release precisely. Dendrimers has emerged as a potential carrier of anticancer drugs due to some unique properties such as mono-dispersity, defined structure, amenability for functionalization using diverse ligands and its low-nanometer size. The dendrimers could be decorated to make them smart enough to carry the drug to the desired locus and release it in a controlled manner. The introduction of stimuli responsive functionality on dendrimers allows the release of payloads in response to a specific trigger only. These triggers could be endogenous in nature (acid, enzyme, and redox potentials) or it could be applied externally (light and temperature). These smart functionalities synergize the targeting of dendrimers and enable dendrimer-based anticancer drug delivery more efficient and safer. This review highlights the potential of stimuli responsive strategy for the controlled release of anticancer drug from dendritic assemblies.

Keywords: Dendrimers; Drug release; Cancer; Drug targeting; Toxicity; Anticancer drug

1. Introduction

Optimizing the outcome of already existing drugs is currently the focusing area of research in view of improving physicochemical, biodistribution and pharmacokinetic properties of the drugs to enhance therapeutic benefit with minimal side-effects. The size manipulation at the molecular level could leads to new intrinsic properties and would be translated as technological innovations for improving the outcome of conventional systems. In field of medicine such technological innovations are termed as nanomedicine. The aim of nanomedicine is to diagnose a disease in its initial stage and to treat it rapidly and specifically, therefore, restrict long-term damage [1]. The field of nanomedicine could be prudently utilized in the management of the world's most deadly disease cancer which is responsible for about 22% of all human deaths annually from non-communicable diseases [2,3].

With hundreds of signaling pathways and multiple causes that respond differently to various treatments, cancer remains an ongoing challenge with enormous health and financial burdens on patients and society. The stage of the cancer is the decisive factor in opting the treatment regimen via surgery, radiation, chemotherapy, biological and hormone therapy. Nonetheless, chemotherapy remains the mainstay option for cancer management and depending on type of cancer and its advancement, it is used as adjuvant with surgery, radiation therapy, or biological therapy [4].

Non-selective biodistribution, low aqueous solubility with poor membrane permeability and rapid clearance, hypersensitivity reactions, and advent of multidrug resistance (MDR) are the major challenges in conventional chemotherapy [5]. Thus, a drug therapy that shows reproducibility in pharmacokinetic behavior and is target specific is sought. A stable, monodisperse, well-defined system could ensure the reproducibility in pharmacokinetic behavior

of a therapy *in-vivo*. This realization leads to the synthesis of hyperbranched polymers termed as dendrimers [6].

2. Dendrimers: An outline

Dendrimers, a class of artificial macromolecules, are nanosize, monodisperse, multivalent polymeric systems with well-defined structure. These unique exciting features ensure a pivotal role of dendrimers in the management of cancer. Structurally, a dendrimer has a treelike molecular construction and comprises of three main architectural components including a core, branching layers (termed as generations) consist of repeating units surrounding the internal core and a multivalent external surface. Generation number (G) signifies the number of focal points from the core towards the surface and is used to determine molecular weight and the number of terminal functional groups [7]. Drugs could either be encapsulated within dendrimers internal cavity or bound to their surfaces through hydrophobic, electrostatic or covalent interactions. The immense potential in this class of molecular construct leads to synthesis of various classes of dendrimers, such as poly(propylene imine) (PPI), poly(amidoamine) (PAMAM), chiral, amphiphilic, micellar, Tecto, Frechet and Janus dendrimers and are also commercially available [8].

Due to highly dense structure, perfect chemical definition and a well-defined number of surface functionalities, dendrimers could be adaptable to multifunctional modifications with valuable flexibility for grafting different chemical moieties on the surface, at the core, or within the structure. The most promising feature of dendrimers is their multi-valency, which presents multiple arrays of ligands to the target bearing multiple receptors. This leads to a greatly increased interface between the dendrimer and the target enhancing affinity and activity [9,10].

2.1 Comparative account of linear polymer, dendritic polymers and dendrimer

Dendritic polymers considered as branched polymeric architectures, are classified into random hyperbranched polymers, dendrigraft polymers, dendrons, and dendrimers based on degree of structural control [11]. In general, branched polymers have sophisticated topological structures and exciting physico-chemical and biological properties. In comparison to their linear analogues, branched polymers have three-dimensional globular structure, lower solution/ melting viscosity, smaller hydrodynamic radius, higher degree of functionality, increased encapsulation capabilities, enhanced solubility and minimal molecular entanglement. Owing to high functionality, dendritic structures, allows dense incorporation of drugs, imaging and targeting agents [12]. Monodisperse nature of dendrimers, in addition, provides reproducible pharmacokinetic behavior as compared to linear polymers, which are generally polydisperse and thus containing varying molecular weighed fractions among a given sample. Also, controlled globular shape of dendrimers other than entangled and coiled structures of linear polymers enhances their biological properties [13]. Furthermore, prospect of surface engineering due their high density of functionalities allow to tune their thermal, mechanical, rheological, solution properties (size, conformation, solubility), and biocompatibility. These features can further improve the biodistribution and pharmacokinetic profile, tendency of crossing biological barriers, blood circulation time and tumor penetration [14-16].

2.2 Toxicologial profile of dendrimers and remedies

The emergence of dendrimers is foresighted as solution to various biomedical difficulties due to distinct features like nanometric size, well-defined structure, narrow polydispersity and large number of surface groups. However, owing their toxic potential including hemolytic toxicity, cytotoxicity, immunogenicity and *in vivo* toxicity, the credentials of their clinical applications is limited. The toxicity of dendrimers is related to surface charge, generation and

size [17, 18]. Various approaches are being utilized to improve the therapeutic index of dendrimers including development of biodegradable and/or biocompatible, surface engineered dendrimers and use of dual drug delivery systems. The biodegradable dendrimers, generally composed by biodegradable repeat units that will eliminate metabolic pathways and thus will not accumulate, therefore considered as non-toxic [19]. Surface engineering masks the cationic charge of dendrimer surface either by neutralization of charge, for example PEGylation, acetylation, carbohydrate and peptide conjugation; or by introducing negative charge on the surface of dendrimers [10]. Dendrimer based hybrid nanocarrier is recognized as a recent approach to minimize the toxicity, improving dispersibility, biocompatibility, payloads and pharmacokinetics of dendrimers as compared to other carriers such as liposomes, nanoparticles, quantum-dots, carbon nanotubes and microsphere [20].

2.3 Dendrimer based drug targeting approach

Dendrimers has emerged as unique polymeric globular nanoparticulate drug delivery system that could be judiciously utilized to tackle the deadliest disease cancer. The inimitable topographical molecular architect encompassing this class of delivery system could allow the delivery of varying nature of anticancer bioactives viz lipophilic or hydrophilic drugs and macromolecules as proteins or RNA. The prospect of multifunctionality owing to multivalency, leads to decoration of their surface by different moieties for varying function to achieve a common goal and this could significantly enhance the efficacy of the transported bioactives. The conventional chemotherapy for cancer management exhibits a lack of selectivity and thus affecting healthy tissues. To realize selectivity, the dendrimers could be functionalized using moieties that would synergistically act to target the tumoral cells and release the payloads at the desired site. The approach of “cellular or secondary targeting” based on moieties that leads to

ligand–receptor-mediated endocytosis or of "tertiary targeting" based on moieties that recognize internal organelles or the use of stimuli-responsive moieties that are responsible for release of bioactives under specific internal or external stimuli, in combination onto a single dendritic structure synergistically act to achieve selective targeting [3,9,20].

3. Dendrimer-based multifunctional theranostics for cancer treatment

The unique features encompassing dendrimers enable their utilization for varied biomedical applications. Early prognosis of the fundamental molecular processes that cause cancer is anticipated from modern imaging technologies. However, the conventionally used contrast agents suffer with short imaging time, renal toxicity and lack of specificity. Dendrimers are now being explored as a platform for surface conjugation of various contrast agents including fluorescent dyes, iodinated contrast agents, and gadolinium (Gd) or radionuclide chelators and for entrapment, stabilization, or assembly of metal (e.g., Au), metal sulfides, and magnetic iron oxide NPs, leading to the formation of a range of contrast agents for different techniques including single mode fluorescence, computed tomography (CT), magnetic resonance (MR), positron emission tomography (PET), and single photon emission computed tomography (SPECT), and dual mode MR/CT, MR/fluorescence, PET/fluorescence, and SPECT/fluorescence. Further, the scope of incorporation of drug into ligand attached dendrimer-based contrast agents could pave the path to design dendrimer-based multifunctional theranostic agents for specific diagnosis and therapy of cancer [21-24].

Though, chemotherapy is currently considered to be the mainstream therapy for cancer management, however due to lack of selective targeting, it would affect healthy cells along with cancerous cells. Tumor physiology allows passive tumor targeting of drug-dendrimer conjugate by enhanced permeability and retention (EPR) effect [25]. In addition to this, molecularly active

targeted delivery of anticancer drugs on specific molecular target, could be achieved by surface functionalization of the dendrimer using various targeting moieties [26]. However, despite considerable efforts towards drug targeting, optimum outcome has not been observed and this may be due to a poor drug internalization and/or inefficient release of drug intracellularly. Efficient therapeutic strategy that could improve targeting and control drug release is currently the area of research in oncology domain. Thus, an efficient targeted drug delivery system should not only be able to achieve preferential accumulation and selective binding to the targeted cells but also been able to endorse cellular internalization, endosomal escape and control drug release [27, 28].

In view of the above, along with targeting ligand, dendritic system encompasses stimuli-responsive controlled release function that is responsive under specific internal or external stimuli. Alike the feasibility of passive targeting to tumor due to EPR effect, the release of the drug at target site could be controlled by exploiting the differential conditions existed at tumor microenvironment. Presence of biochemical gradient between tumor tissue and normal physiological tissue such as pH, redox potential, and enzymes can be exploited as internal stimuli for controlling the release of drugs. Among the external stimuli, use of feature that are responsive to temperature and light are attached onto dendrimers to create externally triggerable systems [29, 30].

Low pH at the extracellular space of solid tumors due to excessive accumulation of lactic acid and lower pH of some intracellular compartments such as the endosomes and lysosomes has driven interest in pH responsive assemblies for cancer therapy. Likewise, interest in redox sensitive carrier develops due to differences in the redox potential between extracellular space and the cytoplasm due to accumulation of reactive oxygen species (ROS) in cancer tissue.

Further, overexpression of various enzymes is observed in cancerous tissue due to augmented metabolic processes and thus could be used for designing enzyme responsive assemblies. Though, the utilization of external stimuli responsive assemblies like light and temperature responsive moieties are being researched for cancer drug delivery, however their utilization is associated with concerns regarding safety and penetration depth [31, 32].

The use of stimuli responsive smart linkers between drug and dendrimer is commonly utilized to control the release of free drug from drug-linker-dendrimer conjugate. The release of the drug in response to the stimuli is due to either reversible and irreversible transformations in the conjugate. However most of the stimuli leads to irreversible cleavage of the linkers. However, the stability and liability of the bond between the dendrimer, linker and drug under physiological conditions and in tumor tissue respectively governs the release behavior of the drug from drug dendrimer conjugate [33].

In addition to this, self-assembled dendritic systems that could disassembled in response to stimuli could also be employed to control dug release. Noncovalent interactions such as hydrogen bonding, π-π stacking and hydrophobic interactions are involved in self-assembly of dendritic systems [34]. The stability and nature of the aggregates are dependent on hydrophilic-hydrophobic proportion, and on external conditions such as temperature and concentration [35, 36].

Further, the class of self-immolative dendrimers is employed to control drug release which upon exposure to a specific trigger allow continuous degradation of their structure into small molecules [37]. In design, self-immolative dendrimers consists of a triggering unit connected to the branched skeleton composed of adaptor units. The adapter units are further attached with drugs as tail units. For controlled release of drugs, stimulation of trigger unit,

initiates rapid disassembly of the branched skeleton, with the subsequent release of all tail-units. “Dendritic amplification” or “Cascade-release” are the term used synonymously for the triggering response that leads to degradation of the conjugate [38-40]. Further, different self-immolative bonds can be used to adjust the degradation rate of the conjugate [41].

This review highlights various stimuli responsive dendritic assemblies utilized for the delivery of anticancer drugs. Different types of internal and external stimuli including acid, reduction potential, enzyme, temperature and light are discussed along with the respective responsive linkers that trigger the release in response to stimuli.

4. pH responsive dendrimers

The existence of pH gradient between tumor microenvironment (pH ~6.5) and normal tissues (pH ~7.4) act as a trigger for the controlled release of payloads from the drug delivery systems. An acid responsive functionality is anchored with such delivery devices, which remains stable in neutral and alkaline environment but gets degraded or hydrolyzed on exposure to acidic environment to release the drug. Further, some intracellular compartments, such as the endosomes and lysosomes have an acidic pH profile (4.5–6.5) that could trigger cytoplasmic drug release from acidic endo-lysosomal compartments [42].

Rapid proliferation effect in tumor tissues results in enhanced glycolysis instead of oxidative phosphorylation resulting in excessive accumulation of lactic acid and this will lead to slight decrease in pH of tumor extracellular environment. This decrease in pH is first observed by Warburg and is termed as Warburg effect [43, 44].

Presence of ionizable functional groups such as amine and carboxylic acid on surface or inner of dendrimer exhibit a pH-dependent release due to disruption of amphiphilicity of the

system. For example, low pH leads to protonation of tertiary amine which decreases the interior hydrophobicity of dendrimer and facilitate the release of payloads at the tumor site [45-47].

The pH gradient, also driven disassembly of assemble structure of amphiphilic dendrimers and thus aid in controlling release of payloads. Under specific pH conditions amphiphilic dendrimers assembled into micelles and these assembled structures tend to disassemble with altered conformation due to pH driven alteration in hydrophilic-lipophilic balance (HLB) [48, 49].

4.1 pH responsive linkers

The use of acid labile linkers could also be utilized in the construction of pH sensitive dendrimer. The acid labile linkers respond to variation in pH, they remain stable in neutral or alkaline pH, but degraded or hydrolyzed at acidic pH. Among pH-responsive linkers, the most frequently employed for anticancer drug delivery via dendrimer drug conjugate are hydrazone, acetal, and cis-acotinyl (**Table 1**) [42, 50].

4.1.1 Hydrazone linkage

Hydrazone linkages are most commonly used pH responsive linkage for designing dendrimer-prodrugs for cancer therapy. The hydrazone linkages hydrolyzed under acidic conditions and remain stable at neutral and alkaline pH [51]. The most common synthetic pathway for hydrazone is the condensation between hydrazines and ketones or aldehydes. Though hydrazones could also be synthesized by reaction between aryl halides and non-substituted hydrazones, and by reaction between aryl diazonium salts and beta-keto esters or acids (Japp-Klingemann reaction) [52].

A ketone or aldehyde group is required for hydrazone formation and thus hydrazine linkage is common with Doxorubicin (DOX)-dendrimer prodrug (**Figure 1**). Antitumor drugs,

without this functional group requires additional modification for conjugation via hydrazone bond. Drugs bearing hydroxyl group such as Paclitaxel (PTX), docetaxel (DTX) and cis-platin could be esterified with acid anhydride or carboxylic acid to obtain active keto sites for hydrazone formation.

Cheng et al, observed the pH-dependent release of DOX by formulating folic acid conjugated poly(ethylene glycol) (PEG)5000-PAMAM(G4) dendrimers using hydrazine linkers. *In vitro* release of DOX from PAMAM-DOX conjugate was evaluated at pH 4.5, 5.5 and 7.4, which was found to be 42, 28 and 8%, respectively. The results revealed the stability of hydrazine linker at pH 7.4 and lability at acidic pH [53]. In another report an amphiphilic linear-dendritic prodrug (mPEG-PAMAM-DOX) for the co-delivery of 10-hydroxy-camptothecin (HCPT) and DOX using acid-labile hydrazine linker was design and revealed acid responsive release behavior. It was observed that as the pH is decrease by 7.4 to 4.5 the release is increased from 5 to 60% revealing pH dependent cleavage of hydrazone linkage [54]. Likewise, She et al, designed mPEGylated peptide dendron-DOX (dendron-DOX) conjugate and demonstrated pH-dependent release of DOX using hydrazone linkage. The *in vitro* release revealed 20 and 80% drug release at pH 7.4 and 5, respectively [55]. Same research group also observed the release of DOX from galactose functionalized PEGylated dendrimer-DOX conjugates having hydrazone linkage. Due to cleavage of pH sensitive hydrazone linker, the release of DOX from the conjugates at pH 5 was much rapid than those at pH 7.4 [56].

Hydrazone linkage was successfully employed for conjugation of DOX to pH-sensitive drug-dendrimer conjugate-hybridized gold nanorods (PEG-DOX-PAMAM-AuNR). Drug release studies revealed that the release of DOX from the conjugate was negligible at pH 7.4, but was boosted considerably at weak acidic pH [57].

A biodegradable hyper-branched HPMA copolymer-DOX conjugate was synthesized with cathepsin B sensitive peptide GFLGK and the anticancer drug DOX was attached to the branched copolymer *via* a pH-responsive hydrazone bond. As compared to traditional copolymers, the biodegradable multiblock HPMA copolymer-drug conjugates resulted in enhanced anticancer efficacy with no obvious side effects [58].

4.1.2 Acetal linker

Chemically an acetal is an organic molecule having a central carbon atom attached to two oxygen atoms by single bond. For ketone derivatives, they are called ketals and are commonly used as protecting groups in organic synthesis and for the design of acid-sensitive linkages [59]. Acetal linkages can be formed by reaction between an aldehyde or ketone and alcohol [60] or between alcohol or phenols and vinyl ether, in the presence of acid catalysts [61]. Acetals are not stable to acidic environment and are very readily hydrolyzed back to the carbonyl and alcohol. However, there are only a few reports of application of acetals as pH-sensitive linkers for anticancer drug delivery using dendritic system.

The pH-responsive micelles based on PEO-dendritic polyester copolymer anchoring an anticancer drug, DOX by acid-labile acetal groups demonstrated the hydrolysis of acetal groups at acidic pH. The DOX was selectively released in tumor vicinity including endosomes and lysosomes [62].

4.1.3 Cis-acotinyl linker

The cis-aconityl, a derivate of natural aconitic acid is commonly used for controlling the release of amine group containing drugs. The interaction between cis-aconitic anhydride and an amine drug leads to a ring opening, which has a carboxylic functionality for conjugating to dendrimer. In mildly acidic conditions, the amide bond undergoes hydrolysis to release the drug.

In an interesting study Yabbarov et al, formulated a conjugate comprising rAFP3D (alpha-fetoprotein) acting as targeting ligand, PAMAM G2 dendrimer and DOX. The cis-aconityl linkage was used to conjugate DOX with PAMAM G2 dendrimer. The *in vitro* release study demonstrated that the release of DOX was found to be pH dependent with 8, 75 and 90% drug release at pH 7.4, 6.0 and 5.5, respectively [63]. Zhong and Da Rocha synthesized PEGylated G3 PAMAM-DOX conjugate by using an acid labile (cis-aconityl) and acid non-labile (succinic) linker. *In vitro* release studies conducted at pH 7.4 and 4.5 revealed 9 and 85% DOX release, respectively [64].

Similarly, Zhu et al, synthesized PEGylated PAMAM G4 dendrimers with different degrees of PEGylation and conjugated with variable amounts of DOX through cis-aconityl and succinic linker and term as PPCD and PPSD prodrugs, respectively. The *in vitro* release study showed negligible amounts of drug released from PPSD prodrug at varied pH values and pH dependent drug release from PPCD prodrug. The cytotoxicity study on murine B16 melanoma cells reveals significant toxicity by PPCD prodrug and negligible toxicity by PPSD prodrug [65].

4.1.4 Boronate ester linkers

Reaction between boronic acid and 1,2-diol or 1,3-diol in aqueous medium leads to the formation of boronate ester, a covalent ester bond. The bond is stable at pH higher than its pKa value but unstable at pH lower than its pKa value. Therefore, boronate ester can be used as pH sensitive linker to construct pH responsive assemblies [66, 67].

Boronate ester bond can be used to prepare bortezomib prodrugs. Catechol-modified PAMAM dendrimer was conjugated to an anticancer drug, Bortezomib via the boronate ester bond. The results revealed the drug release in acidic environment (pH 6.5) and no release at physiological pH [68].

4.1.5 N, O-chelate linker

A pH-responsive mPEGylated peptide dendrimer-linked diaminocyclohexyl platinum (II) (dendrimer-DACHPt) conjugate was prepared by Pan et. al. The DACHPt has a molecular structure, is and activity closely related to oxaliplatin. To achieve pH-sensitive DACHPt conjugation, the N,O-chelate was utilized to link the DACHPt to the dendrimers. The conjugate was pH-responsive and released drug significantly faster in acidic environments (pH 5.0) than pH 7.4. The result revealed that the conjugates suppressed tumor growth better than clinical oxaliplatin without inducing toxicity in an SKOV-3 human ovarian cancer xenograft [69].

5. Redox-responsive dendrimers

Control over release of drug in response to difference in the reduction potential between tumors and normal tissue is frequently employed strategy in cancer therapy. There is highly regulated redox status inside the normal cell balancing the reduced and oxidized species. This balance gets disturbed in cancerous cells, which leads to accumulation of ROS and results in oxidative stress. To overcome oxidative stress, cells recruit ROS scavengers such as glutathione (GSH) and vitamins C and E. The significant difference (about 4-fold) in GSH concentration intracellularly (2-10$\times10^{-3}$ M) and extracellularly (2-20 μM) in cancerous tissue have made GSH responsive assemblies most explored for reductive responsive drug delivery systems [70-72]. Further, a specific reducing enzyme, gamma-interferon-inducible lysosomal thiol reductase (GILT) modulates the redox potential of endosomal compartment in the co-presence of a reducing agent such as cysteine [73-75].

The frequently used redox-reponsive linker for dendrimer drug conjugate is disulphide linker. The elevated GSH mediates disulfide bond cleavage reactions via reduction or dithiol-disulfide exchange process (**Figure 2**). Besides disulfide bonds, diselenide or ditellurium bonds

are also used as redox responsive linkers. The diselenide bond is more sensitive than disulphide bond towards stimuli as the cleavage energy of diselenide bonds is lesser as compared to disulfide bonds [76-78].

A novel stimulus responsive conjugate of dendrimer and gold nanoparticle (GNP) was developed for the delivery thiolated anticancer drugs by Wang et al. Dendrimer-encapsulated gold nanoparticles (DEGNPs) were synthesized and thiolated anticancer drugs are attached through the Au-S linkage. The conjugate exhibited an "Off-On" release behavior in the presence of thiol-reducing agents such as glutathione and dithiothreitol. The developed conjugate showed much reduced cytotoxicity as compared to the free anticancer compounds [79].

A new class of disulfide cross-linked G2 PAMAM dendrimers was prepared as non-viral gene carrier to enhance transfection efficacy and to reduce cytotoxicity. Disulfide containing linker 3,3′-dithiodipropionicacid-di(N-succinimidyl ester), (DSP) was used to cross-link G2 PAMAM dendrimers to form supra-molecular structures (G2DSPs). The cross-linked conjugate was degraded due to disulfide bond reduction after gene transfection and this regulated the release of DNA in a controlled manner [80].

In a recent study a redox responsive peptide conjugated tumor targeted nano vehicle (PSPGP) composed of branched PEG with G2 dendrimers was synthesized for co-delivery of PTX and siTR3 for treatment of pancreatic cancer. The assembly was conjugated with PTP (plectin-1 targeted peptide, NH_2-KTLLPTP-COOH), a biomarker for pancreatic cancer. Redox-responsive disulfide bonds were used to link the PTX and siTR3 to the conjugate. The complex showed inhibition in tumor growth and promoted cancer cell apoptosis [81].

Lim et al, synthesized 3 conjugates of PTX with PEGylated triazine dendrimer. The dendrimer construct 1 includes an ester linker, whereas dendrimer construct 2 and 3 include a

disulfide linker. Cytotoxicity studies using an MTT-based assay and PC-3 cells revealed IC_{50} values of 4.5 and 29 nM for free PTX and construct 1, respectively and increased in construct 2 and 3 from 74 to 26 nM and 13nM in the presence of 1 mM glutathione and 1 mM dithiothreitol, respectively [82].

Reduction-responsive amphiphilic dendritic copolymer (TPP-S-S-G3) with disulfide-linkages between dendrimer (PEG-G3-OH) and porphyrin (TPP, photosensitizers) for the combined chemotherapy and photodynamic therapy (PDT) was developed. The copolymer self-assembled into micelles in aqueous solution. The results showed fast uptake and release of DOX-loaded TPP-S-S-G3 micelles by MCF-7 cells [83]. Nguyen et al, studied Heparin (Hep) conjugated to PAMAM G3.5 (P) via redox-sensitive disulfide bond (P-SS-Hep). The dendrimer complexes were found to promote redox-sensitive drug release intracellularly. In the cancer cells the disulfide linkage cleaved and enabled the release of drug. Hence, providing evidence of potential of redox sensitive nanocarriers in cancer chemotherapy [84].

Dual responsive PAMAM dendrimers that responded to variation in reduction potential and pH have been used for the delivery of DOX. The redox-responsive functionality is imparted using disulfide linkage between PAMAM dendrimers and PEG with DOX loaded into the hydrophobic core of the conjugates. The release study demonstrated redox and acid trigger release behavior of DOX [85]. For tumor-targeted drug delivery an asymmetric bow-tie PAMAM dendrimer (ABTD) scaffold has been developed using disulfide unit as self-immolative linker. The results revealed a remarkable selectivity of ABTD scaffold to cancer cells as compared to human normal cells and demonstrated reduction responsive release behavior [86].

A GSH-triggered self-immolative dendritic prodrug has been designed for cancer therapy. The assembly comprised an anticancer drug Camptothecin (CPT), a reduction cleavable

linker (2,4-dinitrobenzenesulfonyl, DNS) and a near infrared (NIR) fluorescent dye (dicyanomethylene-4H-pyran, DCM). Cleavage of the DNS linker in the presence of GSH released the drug and activated NIR fluorophore, which could aid to track the released drug [87].

To develop highly efficient and safe gadolinium (Gd)-based MRI contrast agents with minimum bio-accumulation and least detrimental effect on the body, Guo et.al, develop biodegradable Gd-based polymeric contrast agents with a biocleavable disulfide linker. Biodegradable poly[N-(1,3-dihydroxyprpyl) methacrylamide] copolymers (pDHPMA) were synthesized and small molecular Gd-chelate (Gd-DOTA) was conjugated onto the copolymer backbone through a sulfide bond or a GSH-sensitive cleavable disulfide bond to produce two novel Gd-mCAs (pDHPMA-Cy5.5-DOTA-Gd or pDHPMA-Cy5.5-SS-DOTA-Gd) for tumor diagnosis. The developed contrast agents demonstrated enhanced relaxation efficiency, improved pharmacokinetics and better passive targeting through EPR effect as compared to Gd-diethylenetriamine pentaacetic acid (DTPA-Gd) [88].

6. Enzyme-responsive dendrimers

Changes in the level and activity of various enzymes are observed in cancer etiology. As, cellular metabolic processes are augmented in cancer tissue, the enzymes that regulate these processes are commonly overexpressed. This dysregulation of their expression is considered as characteristic feature of the cancer and is utilized as a tool in diagnostics. Along with diagnostics, such dysregulation is utilized in managing the disease condition by programming the drug delivery system for active targeting and to control the release of drugs. The on-demand drug release, governed by enzyme is designed by integrating specific linkers that can be recognized and degraded by the enzymes overexpressed in the extracellular or intracellular environment of the tumor [89, 90]. A variety of enzymes are found to be upregulated in tumor tissues including

cathepsins, matrix metalloproteases (MMPs), hyaluronidase, azoreductase, phospholipase and many more [91].

Further, the advent of enzyme responsive self-immolative dendrimers as molecular amplifiers has translated the release of drug on enzymatic stimulation. Incorporation of drug molecules as the tail units and an enzyme substrate as the trigger in self-immolative dendrimers, generated a prodrug unit that was triggered upon a single enzymatic cleavage. The enzymatic trigger commonly utilized for the same is 38C2 antibody, penicillin-G-amidase and β-galactosidase [92, 93].

Cathepsins, a group of proteolytic enzymes predominantly located in endo/lysosomal vesicles, are involved in the degradation of extracellular matrixes (ECM) of the tumor tissue and thus contributing to infiltration of the tumor cell. Out of various cathepsins, cathepsin B is one of most explored lysosomal proteases due to its high expression in various types of cancers including prostate, breast, lung, brain, endometrium and colorectum. Invasive and metastatic cancers are the results of abnormal regulation of cathepsins [94, 95].

Lee et al, synthesized dendrimer-methoxy PEG (MPEG)-DOX conjugates using a cathepsin B-cleavable peptide, glycyl phenylalanyl leucyl glycine tetra-peptide (Gly-Phe-Leu-Gly) for anticancer drug targeting (**Figure 3**). The results revealed improved anticancer activity in an *in vivo* CT26 tumor xenograft model *i.e.* the volume of the CT26 tumor xenograft was significantly inhibited [96].

Cathepsin B-cleavable peptide (Gly-Phe-Leu-Gly) was successfully used to develop a novel enzyme-responsive PEGylated lysine peptide dendrimer-gemcitabine (GEM) conjugate (Dendrimer-GEM) based nanoparticle. The results indicated suppressed relative tumor volumes

(86.17±38.27%) and a 2-fold higher value of tumor growth inhibition (~90%) than GEM, establishing enhanced antitumor efficacy without obvious systemic toxicity [97].

In another study cathepsin B-cleavable peptide was utilized by Zhang and coworkers to develop mPEGylated peptide dendrimer-DOX (dendrimer-DOX) conjugate-based nanoparticles, which demonstrated significantly high antitumor activity and substantially reduced DOX-related toxicities [98]. Similar peptide along with a pH-sensitive hydrazone bond was exploited by Chen et al, for the preparation of a novel pH/enzyme sensitive dendritic polymer-DOX conjugate for cancer treatment. The result revealed high accumulation of DOX into tumors due to prolonged blood circulation time. *In vivo* studies revealed better antitumor efficacy of the conjugate in comparison with free DOX [99].

Wang et al, designed an enzyme-stimuli nanogel based on G4 PAMAM dendrimers using elastase cleavable bond (Ac-arg-ala-ala-asp-D-tyr-cys-NH_2) (RAADyC). Neutrophil elastase (NE) is detected in different types of cancers, and its concentration is associated with the cancer stage, grade, and the survival [100].

Azoreductase, an enzyme over-expressed in hepatocellular carcinoma cells, can work as a trigger to induce drug release. Medina et al, synthesized a series of aromatic azo-linkers (L1-L4), which were used to conjugate DOX to G5 PAMAM dendrimers. To study the effect of electronegativity on susceptibility to cleavage by azoreductase enzymes, these linkers are incorporated with electron-donating oxygen (O) or nitrogen (N) groups. Results revealed the release of 4-8, 17, 60, and 100% of the conjugated DOX molecule from dendrimers having linkers L1 to L4, respectively. Increase in electronegativity increases susceptibility to cleavage by azoreductase enzymes [101].

Phospholipase C (PLC) enzyme, an important regulator of membrane phospholipid metabolism is found to be overexpressed in many cancers and participates in cancer cell progression and differentiation [102,103]. Zhang et al, synthesized enzyme-responsive phosphoramidate (PAD) dendrimers for delivery of DOX. The dendrimers were degradable in the presence of PLC but found to be stable in phosphate buffer saline (PBS). The phosphite ester bonds in PAD dendrimers is degraded by PLC. The results revealed improved therapeutic efficacy of the conjugate with reduced toxicity in athymic nude mice bearing xenografts of MCF-7/ADR breast cancer [104].

A dendritic prodrug with an anticancer agent camptothecin (CPT) and a trigger that allowed activation by penicillin-G-amidase was designed and synthesized. Cell-growth inhibition assays demonstrated that the toxicity of the dendritic prodrug was found to be dependent upon incubation with penicillin-G-amidase [105]. Shamis et al, prepared a self-immolative assembly for synergistic combinational therapy in cancer utilizing DOX and CPT as tail units and a catalytic antibody 38C2 cleavable retro-aldol retro-Michael focal trigger [106].

In an effort to improve therapeutic index of an anti-cancer drug, gemcitabine (GEM), a stimuli-responsive dendritic polyHPMA copolymer was designed and synthesized GEM (Dendritic polyHPMA-GEM) prodrug via one-pot synthesis of RAFT polymerization by Dai and coworkers. GEM was conjugated onto the dendritic polymeric carrier via an enzyme-responsive linker glycyl-phenylalanylleucyl-glycine tetra-peptide (GFLG), which was found to be stable in blood circulation system and degraded in the presence of Cathepsin B only. The results revealed that the designed stimulus-responsive dendritic copolymer-GEM prodrug may a safe, effective and enzyme-responsive anticancer agent [107].

Polymer-drug conjugates has significantly improved the anti-tumor efficacy of chemotherapeutic drugs and alleviated their side effects. In this regard a biodegradable diblock N-(1,3-dihydroxypropan-2-yl) methacrylamide (DHPMA) copolymer-DOX conjugate (a self-aggregation-induced nanoprodrug) via one-pot of RAFT polymerization and conjugate chemistry was developed. Notably, the nanoprodrug had a significantly prolonged blood circulation time with an elimination half time of 9.8 h. It displayed high accumulation within tumors, and improved e *in vivo* therapeutic efficacy against 4T1 xenograft tumors compared to free DOX. The authors demonstrated that the diblock pDHPMA-DOX nanoprodrug with a controlled molecular structure exhibited an enhanced antitumor efficacy against 4T1 breast tumors through the inhibition of cell proliferation and antiangiogenic effects and alleviated side effects, showing a great potential as an efficient and safe anticancer agent [108].

7. Temperature-responsive dendrimers

Among external stimuli, temperature trigger drug release has shown significant potential. However, the use of temperature as a trigger requires external heating methodology that can heat the tumor area locally and thus respond to temperature variation [109]. Modification of dendrimer surfaces with oligo- and poly-ethylene oxide-based functionality endow them with temperature-sensitive characteristics [110].

There is an inverse relationship between aqueous solubility and temperature for temperature sensitivity functionalities. As temperature is increased the degree of hydrogen bonding between the temperature sensitive moieties and water decreases, and this will leads to phase separation. Lower critical solution temperature (LCST) or the cloud point is the phrase used demark such phase transition and is specific for a moiety [111]. Most commonly used thermo-responsive material includes PEG and poly(N-isopropylacrylamide) (pNIPAM). These

functional groups become hydrated due to hydrogen bonding with water and the application of temperature trigger breakdown of these weak interactions causing them to lose its hydrophilicity [112,113].

Thermosensitive pNIPAM polymer-conjugated PAMAM dendrimer has efficiently delivered Malloapelta B (Mall B) against HepG2 cancer cell proliferation. The conjugate showed high encapsulation of Mall B and demonstrated slow controlled release, which significantly inhibited HepG2 cancer cell proliferation [114]. Wu et al, synthesized G4 thermosensitive dendrimers based on oligo (ethylene glycol) (OEG) conjugated with an antitumor agent, GEM. The prepared dendrimers were compared with that of GEM-conjugated PAMAM dendrimers. The GEM-OEG based dendrimers exhibited thermal responsive release behavior and better tumor accumulation and penetration than the GEM-conjugated PAMAM [115].

A temperature responsive dendrimer conjugate was prepared for gene silencing through intracellular small interfering RNA (siRNA) release. The pNIPAM and phenylboronic acid were conjugated with PAMAM dendrimer for the design temperature responsive system. The phenylboronic acid improves the stability and cellular uptake while pNIPAM is responsible for temperature responsive behaviors at lower critical solution temperature. The results revealed that gene silencing efficacy was significantly increased by cool treatment after its cellular uptake with minimal toxicity [116].

Though, temperature-sensitive materials for dendrimer drug conjugate is numerous, a few are potentially utilized for temperature-responsive drug release. This is probably due to difficulty in controlling the release of the drug during phase transition and the safety concerns of the temperature-sensitive polymers above LCST for *in vivo* applications. Further, it is very difficult to heat localized tissues without hurting normal tissues.

8. Light-responsive dendrimers

As external stimuli, light is most explored due to some obvious advantages such as non-invasiveness and prospect of temporal and spatial accuracy. The principle governing the release of drug from dendrimers using light as a stimulus is based on- (i) the absorption of light by photosensitive ligands that would trigger reversible physical changes (e.g., trans-cis isomerization) and cause release of the encapsulated drugs and (ii) the absorption of light by photosensitive ligands causes irreversible cleavage reaction. The most common photosensitive ligands for the former are azobenzene derivatives and for later are o-nitrobenzyl ether (or ester) derivatives grafted on the surface of dendrimers [117].

The commonly used light triggers includes ultraviolet (UV) (200-400 nm), visible (400-700 nm) or near-infrared (NIR) (700-1000 nm) light. However, UV and visible light usage gives poor tissue penetration as well as leads to phototoxicity. NIR light irradiation has deeper tissue penetration with the penetration depth of up to 2 cm with less phototoxicity and thus preferred. Nevertheless, NIR light has inherent low energy and due to this two-photon excitation technique would be considered ideal for photobiological applications using NIR light irradiation or the application of upconversion nanoparticles, which can convert adsorbed NIR light to UV irradiation (**Figure 4**) [118-120].

Choi et al, designed folic acid conjugated G5 PAMAM dendrimer and photocaged DOX using the photocleavable group ortho-nitrobenzyl (ONB) (**Figure 5**). The *in vitro* cytotoxicity studies using KB cell-based assay revealed release of DOX and cytotoxicity on exposure to UV light [121]. Similarly, in another study same group of researchers designed targeted PAMAM dendrimer for the delivery of methotrexate (MTX). The *in vitro* cytotoxicity study using KB cell-based assay demonstrated MTX release through a light-controlled mechanism following

exposure to UV light [122]. Sun et al, designed DOX loaded Janus-type dendritic structure by linking a hydrophobic dendron (diazonaphthoquinone (DNQ)-decorated G3 PAMAM) and a hydrophilic dendron (lactose (Lac)-decorated Gm PAMAM dendrons). The DNQ, undergoes a Wolff rearrangement to form a ketene, on exposure to light. These Janus dendritic structure gets self-assembled into micelle in aqueous solution and gets disassemble on exposure to NIR light. The results presented a photo-triggered cytotoxicity and revealed doubling of DOX release on irradiation to NIR [123].

Coumarin, a natural dye, with high two-photon cross sections is utilized as photocages for the light responsive release of chemotherapeutic drugs [124]. Wang et al, synthesized a light responsive construct for the co-delivery of 5-Flurouracil (5-Fu) TRAIL plasmid for cancer therapy. The anticancer moieties were loaded on amphiphilic G1 dendrimer-coumarin conjugate (G1-CM). Coumarin acts as photoresponsive group and on exposure to light leads to degradation of the assembled structure and exhibits a light-responsive drug release profile [125].

Thioacetal ortho-nitrobenzaldehyde TNB(OH) photolinker was utilized for the construction of TNB-caged DOX conjugates. The constructed caged conjugates are then integrated with 2 folic acid functionalized nano-assemblies. First is, G5 PAMAM dendrimer and second is upconversion nanocrystal (UCN) conjugate with protoporphyrin IX (PPIX) as cytotoxic photosensitizer. Cellular toxicity studies in KB carcinoma cells revealed that each nano-assembly exhibit cytotoxicity on exposure to UV or NIR (980 nm) [126]. However, despite various obvious benefits of light as a stimulus for drug release, its application is limited in the treatment of solid tumor due to the ambiguity regarding penetration depth, irradiation time and effective area.

9. Smart dendrimers in gene delivery

An alternative strategy to traditional radiotherapy and chemotherapy, gene therapy is now recognized as a potential therapeutic modality for cancer treatment. Gene therapy has been extensively explored for the management of cancer, as approximately 65% of the clinical trials in gene therapy have been designed at the treatment of various types of cancers [127]. To realize gene transfer complex cellular and tissue barriers must be overcome without disrupting vital regulatory mechanisms to deliver the tailored therapeutic gene for augmentation or suppression or repair, using a vehicle called vector [128].

In addition to carrier of chemotherapeutic agent and contrast agent in molecular imaging for cancer treatment and prognosis, dendrimers are also considered as non-viral vector for gene therapy. In contrast to viral vectors, dendrimers as a non-viral vector offer distinct advantage including target-cell specificity and resistance to repeated administration. Further, the biodegradable dendrimers are preferred for gene delivery as compared to the non-degradable dendritic vectors, due to their reduced toxicity and degradability. The PAMAM dendrimers, dendritic polyglycerols and peptide dendritic polymers are the commonly investigated vectors for gene therapy [129, 130].

Alternatives to viral-mediated gene delivery, dendrimers are now being largely investigated as an effective non-viral mediated gene delivery system. Though, viral vectors have high transfection efficacy but are accompanied by high immunogenicity, cytotoxicity and production problems. Owing to possibility of multifunctionality, dendrimers are perceived as non-viral vector that can overcome these limitations [131]. However, cationic dendrimers is associated with serious toxicity and thus a key challenge in clinical gene therapy is to prepare dendritic vector with high transfection efficacy and low toxicity. Fluorinated dendrimer, a new class of non-viral gene carriers exhibits interesting physicochemical properties, with efficient

cellular internalization and less toxicity [132,133]. A structure-activity relationships (SAR) study for DNA and siRNA delivery based on different dendrimer generations and fluorination degrees reveals that fluorination significantly improves the transfection efficacy of G4-G7 PAMAM dendrimers. Fluorination on G5 dendrimer yields the most efficient polymers in gene delivery, and the transfection efficacy of fluorinated dendrimers depends on fluorination degree. All the fluorinated dendrimers cause minimal toxicity on the transfected cells at their optimal transfection conditions [134].

A series of fluorodendrimers was synthesized, by reacting PAMAM dendrimers with heptafluorobutyric anhydride, as non-viral gene vectors. The synthesized conjugate self-assembled to form uniform polyplexes with promising properties at a low nitrogen-to-phosphorus ratio and have low charge densities and relatively weak DNA associations. Uniform polyplexes ensures reproducible gene transfection. A low charge density indicates low cytotoxicity and weak DNA association, which is beneficial for efficient DNA unpacking in the cytoplasm [135].

The interaction of G5 PAMAM dendrimers with perfluoro acid anhydrides resulted in the development of fluorinated dendrimers with high transfection efficacy and low toxicity. The study revealed that fluorination of the dendrimers improved the transfer across cell as well as the endosome/lysosome membrane facilitating endosomal escape. Further, this class of dendrimer has shown to form polyplexes with genes at low nitrogen to phosphorus (N/P) ratios to minimize the toxicity on the transfected cells [136].

A stimulus-responsive fluorinated bola-amphiphilic dendrimer bearing ROS-sensitive thioacetal in the hydrophobic core and positively charged PAMAM dendrimer at the terminals was synthesized for the delivery of siRNA in cancer cells. The conjugate combine the

advantageous delivery features of both lipid and dendrimer as a non-viral vector. The result revealed that the conjugate capable of interacting and compacting the negatively charged siRNA into nanoparticles to protect the siRNA and promote cellular uptake [137].

A heptafluorobutyric acid modified G4PAMAM dendrimer (G4-F735) has been used as a nonviral vector to deliver plasmid encoding TNF-related apoptosis-inducing ligand (pTRAIL) gene for cancer treatment to achieve both excellent transfection efficacy and low toxicity. The results revealed much higher TRAIL gene transfection efficacy than a series of transfection reagents including poly(ethylene imine) (PEI), SuperFect and Lipofectamine 2000 and exhibited minimal toxicity *in vitro* [138].

For the investigation of fluorous effect on transfection efficacy and cytotoxicity, Wang and Cheng synthesized a series of fluorobenzoic acid (FBA)-modified dendrimers as non-viral gene vectors. The results demonstrated that the transfection efficacy increases with increasing number of fluorine atoms on the aromatic rings. The modified dendrimers were found to be superior as compared to the polymer-based and lipid-based commercial reagents such as SuperFect, PolyFect, and Lipofectamine 2000, respectively. Fluorination on the aromatic rings significantly improves the transfection efficacy of benzoic acid-modified dendrimers [139].

In a study fluorodendrimer was prepared by reacting G2 PAMAM dendrimer with heptafluorobutyric anhydride for the co-delivery of fluorinated anticancer drugs (sorafenib or 5-Fu) and therapeutic genes (TRAIL plasmid) in synergistic cancer therapy. The results revealed high drug loading and gene transfection efficacy with minimal toxicity [140].

10. Conclusion

The synergy that exists between experimental and theoretical studies opens new avenues for the use of dendrimers as versatile drug delivery systems. The possibility of diverse functionalization

on dendritic structure paves the path for delivery of drugs in spatial-, temporal- and dosage-controlled fashions for cancer therapy. The use of stimuli responsive smart linkers facilitates the delivery of payloads in a controlled manner on specific triggers. The incorporation of pH and redox responsive systems into dendrimers, has attracted significant interest. Various functional groups have been utilized in dendritic assemblies such that a pH sensitive linker would provide stability to the assembled nanostructure stable at neutral pH 7.4, but would respond to a lower pH. The use of pH-, redox-, enzyme-, thermal- and light-responsive ligands potentiate the target functionalized dendrimers in delivering anticancer bioactives in an efficient and safer manner.

Declaration of interests

None

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Table legend:

Table 1. Acid-responsive chemical bonds and corresponding degradation products under acidic environment [Modified and reproduced with permission from Ref 42]

Figure legends:

Fig 1. Dendrimer-doxorubicin conjugate via hydrazone linkage [Modified and reproduced, with permission from Ref 32]

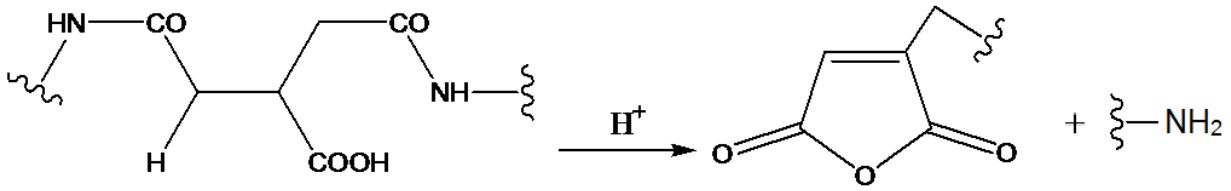
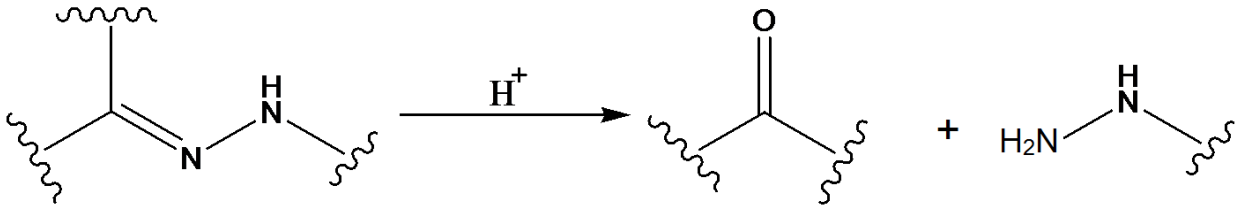
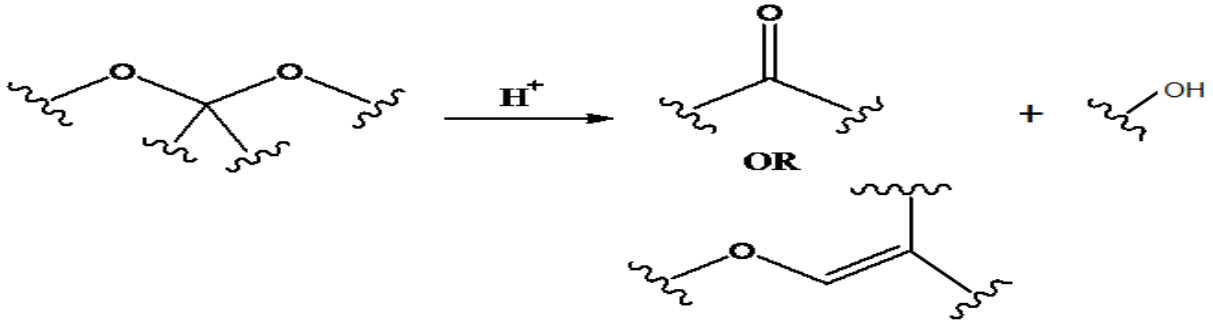
Fig 2. Cleavage of disulfide bond between dendrimer–drug conjugates via GSH [Modified and reproduced, with permission from Ref 32]

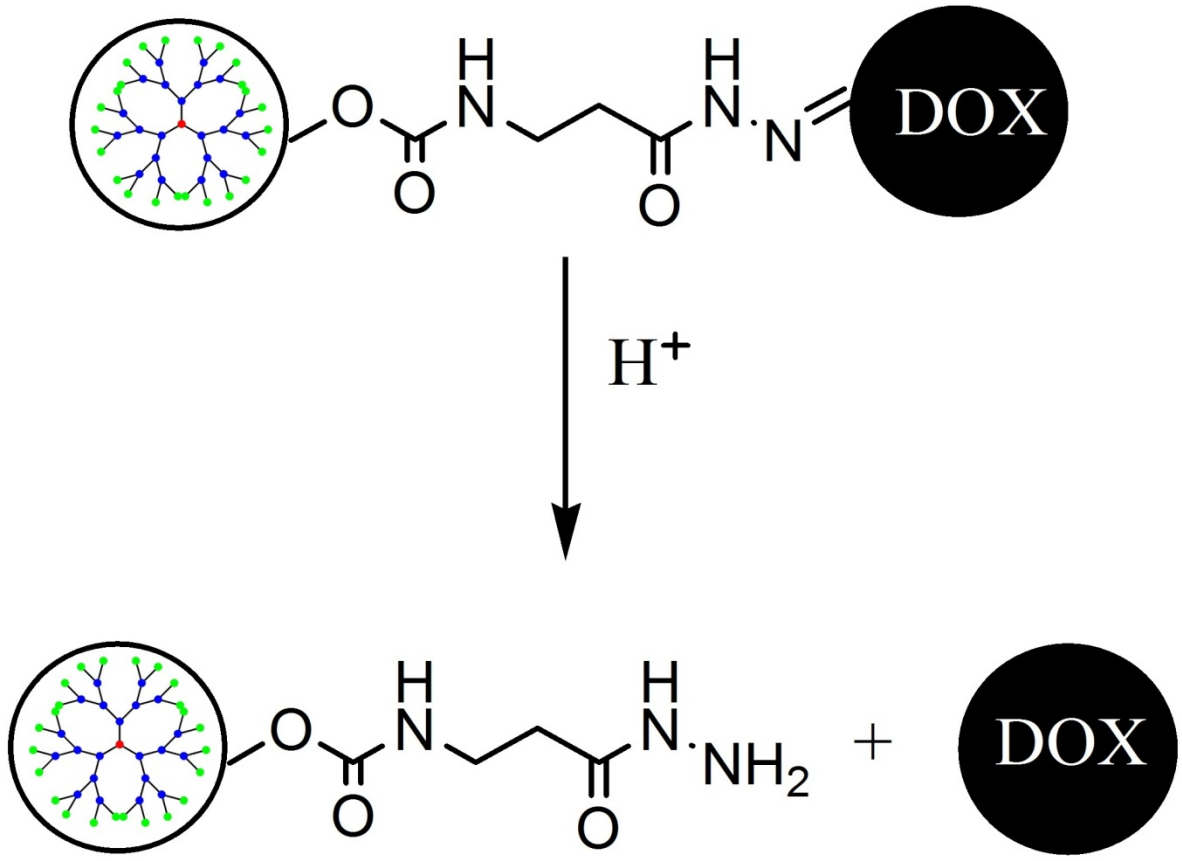
Fig 3. Cleavage of glycyl phenylalanyl leucyl glycine tetra-peptide (GFLG) between dendrimer-doxorubicin conjugates by Cathepsin-B [Modified and reproduced, with permission from Ref 32]

Fig 4. Photo-cleavable groups: (a) Ortho-nitrobenzyl (ONB) group and (b) Coumarin [Modified and adopted from Ref 118].

Fig 5. Cleavage of ortho-nitrobenzyl (ONB) linker in dendrimer-doxorubicin conjugate upon UV light irradiation [Modified and reproduced, with permission from Ref 32]

Table 1. Acid-responsive chemical bonds and corresponding degradation products under acidic environment [Modified and reproduced with permission from Ref 42]

Acid-responsive chemical bond	Structure	Degradation products	Reference
Cis-Aconityl			[42]
Hydrazone			
Acetal			



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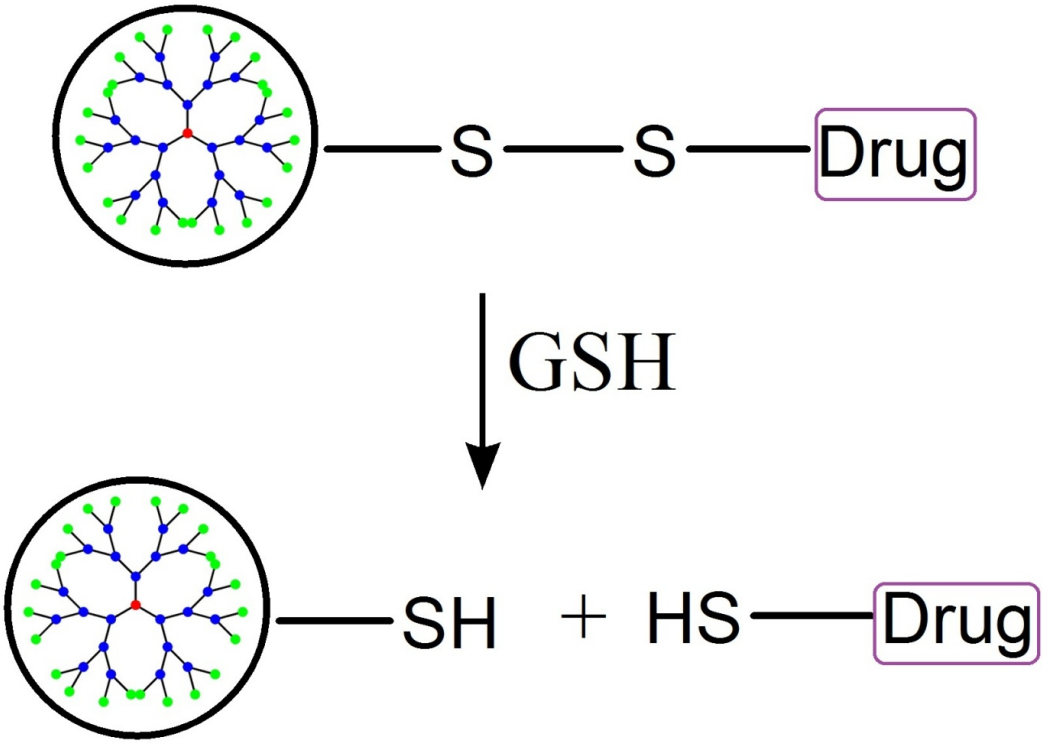

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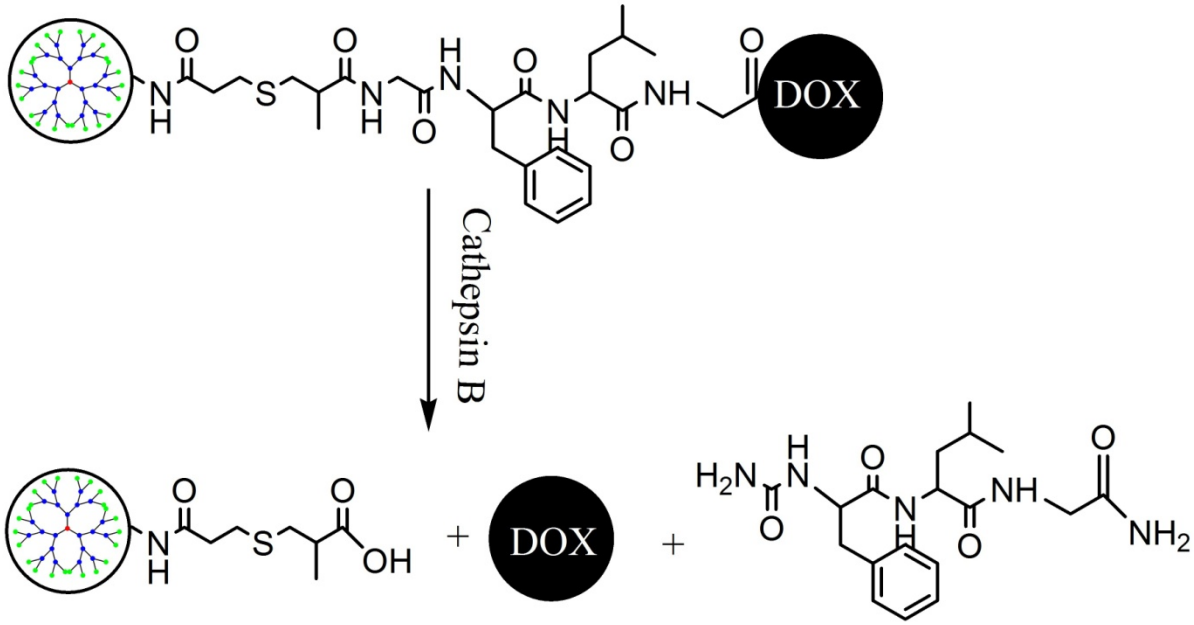

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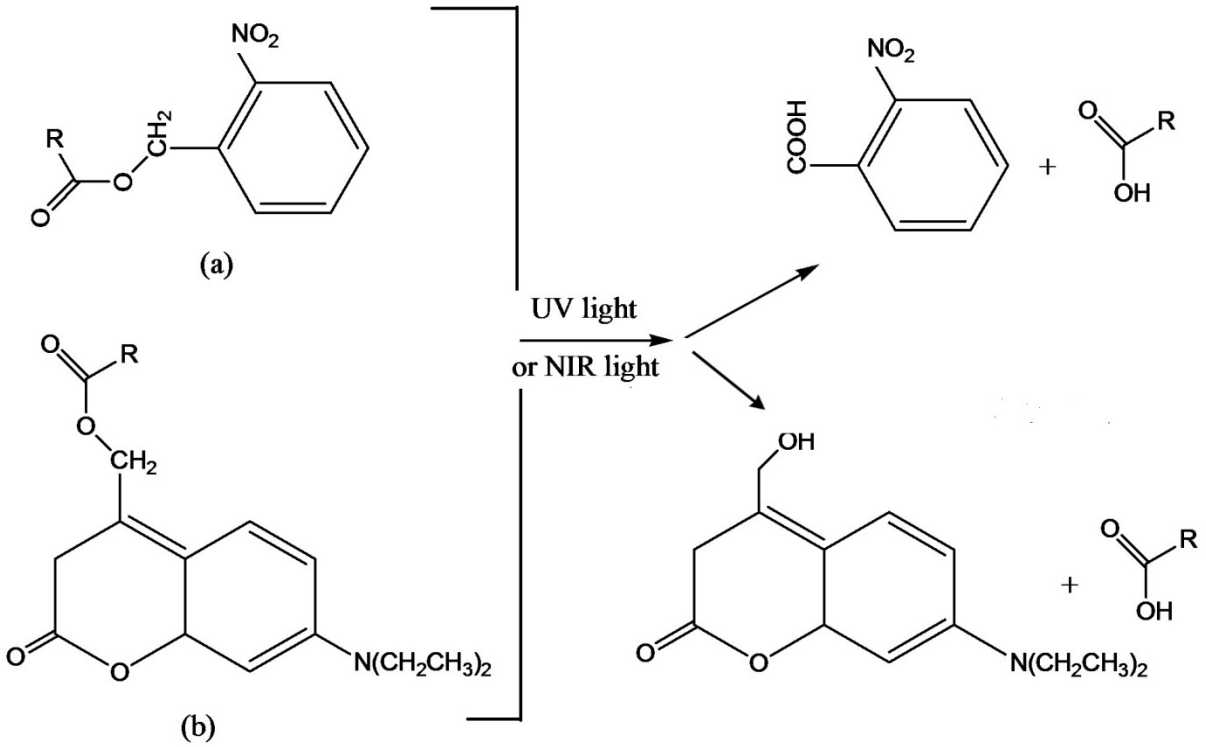

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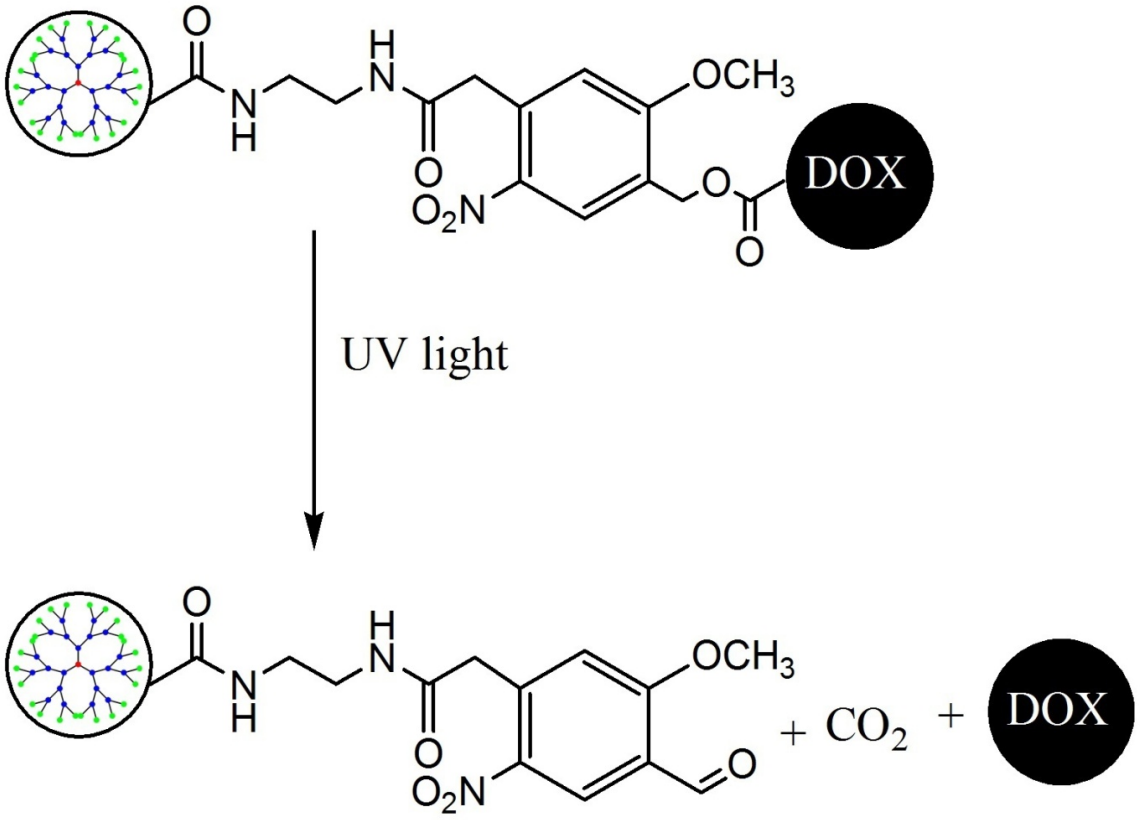

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